GENERAL PROGRAMME

Saturday Sept 16th

18:00	*Welcome reception* (Old Medical School)*

Sunday Sept 17th

08:30 - 18:00	Symposium “Hearing rehabilitation and Innovative Inner Ear Therapy"

Monday Sept 18th

08:45 - 17:45	IEB-2006 Sessions I to IV and Posters
19:00	*Mayor Reception* (City Hall)*

Tuesday Sept 19th

09:00 - 16:00	IEB 2006 sessions V to VII and Posters
16:00 - 16:15	Spoendlin Junior Award
16:30 - 17:30	IEB Business Meeting
19:30	*Gala Dinner** (Opera Comédie)*

Wednesday Sept 20th

08:45 - 12:00	IEB : Sessions VIII and IX
12:00	End of the Meeting

** Free of charge, but upon reservation (registration form) only*

*** Tickets only available online (registration form)*

SYMPOSIUM

Hearing Rehabilitation and Innovative Inner Ear Therapy

Sunday, 17 September

Programme

08:30-08:45		Welcome*:* JL Puel, R Pujol and official representatives for University Montpellier 1, INSERM, Montpellier-Agglomération and Région Languedoc-Roussillon
		Session I : ***Hearing Rehabilitation*** Moderators : J Magnan, M Mondain
08:45 - 09:10	S1	Hearing aids and brain plasticity Collet L, Veuillet E, Thai-Van H
09:10 - 09:30	S2	New developments in hearing aid technology Chalupper S
09:30 - 10:00	S3	Implantable middle ear devices Lefebvre PP
10:00 - 10:25	S4	New trends in cochlear implantation Uziel A
10:30 - 11:00		Pause
		Session II : ***Pharmacology*** Moderators: P Gil-Loyzaga, R Pujol
1100 - 11:30	S5	From brain molecular pharmacology to clinical applications Bertrand D
11:30 – 11:55	S6	Local therapy of hearing loss and tinnitus Puel JL
11:55 – 12:20	S7	Mechanisms of auditory hair cell death in response to oxidative stress initiated damage and otoprotective strategies to preserve hearing Van De Water TR
12:20 - 12:45	S8	Gene expression in cisplatin ototoxicity and protection with p53 inhibitor Salvi R, Ding D, Jiang H
12:45 - 14:30		Lunch

Session III : *Cell and gene therapy*
Moderators: N Trigueiros-Cunha, A Uziel

14:30 - 14:55	S9	Cellular therapy for inner ear disorders Ryan AF, Pak K, Mullen LM
14:55 - 15:20	S10	Strategies to regenerate hair cells: identification and differentiation of progenitors Malgrange B
15:20 -15:45	S11	Developing stem cell-based therapies for deafness: Isolation and differentiation of human fetal auditory stem cells (hFASCs) Chen W, Johnson S, Marcotti W, Moore H, Andrews P, Rivolta MN
15:45 -16:10	S12	Probing the structure and function of the tectorial membrane in tectorin mutant mice Richardson G, Russell IJ, Legan PK, Lukashkina V, Goodyear RJ, Lukashkin A
16:10 - 16:40		Pause

Session IV : *Cell and gene therapy (continued)*
Moderators: A Martini, JL Puel

16:40 - 17:05	S13	Re-activation of developmental signaling for repair of mature inner ears Batts SA, Crumling MA, Miyazawa T, Kim YH, Izumikawa M, Shoemaker CR, Johnson MD, Qazi T, Dolan DF, Pfingst BE, Raphael Y
17:05 - 17:30	S14	Human hereditary deafness: from genes to the underlying pathogenic processes Delmaghani S, Roux I, Safieddine S, del Castillo F, Nouvian R, Grati M, Avan P, Moser T, Petit C

Closing lecture
Moderator: R Pujol

17:30 -18:00	S15	The new era of hearing habilitation Rubel EW

IEB-2006 WORKSHOP

Programme

Monday, 18 September

08:45 - 09:00	Welcome and Workshop opening
09:00 - 10:30	Oral session I – *Neurotransmission* (O1 - O6)
10:30 - 11:00	**Pause**
11:00 - 12:30	Oral session II – *Endolymph homeostasis* (O7 - O12)
12:30 - 13:30	**Lunch**
12:30 - 14:30	Poster session (Presentation by authors of odd number posters)
14:30 - 16:00	Oral session III – *Rescue* (O13 - O18)
16:00 - 16:30	**Pause**
16:30 - 17:45	Oral session IV – *Ototoxicity and physiopathology* (O19 - O23)
19:00	*Mayor Reception* (City Hall)*

Tuesday, 19 September

09:00 - 10:30	Oral session V – *Mechanisms of development and repair* (O24-O29)
10:30 - 11:00	**Pause**
11:00 - 12:30	Oral session VI – *Deafness genes* (O30 O35)
12:00 - 13:30	**Lunch**
12:30 - 14:30	Poster session (Presentation by authors of even number posters)
14:30 - 16:00	Oral session VII – *Micromechanics* (O36 – O41)
16:00 - 16:15	Spoendlin Junior Award
16:15 - 16:30	**Pause**
16:30 - 17:30	IEB Business Meeting
19:30	*Gala Dinner** (Opera Comédie)*

Wednesday, 20 September

08:45 - 10:15	Oral session VIII – *Cell and gene therapy* (O42 - O47)
10:15 - 10:45	**Pause**
10:45 - 12:00	Oral session IX – *Ionic signalling* (O48 – O52)
12:00	End of the Meeting

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IEB-2006 WORKSHOP

Detailled programme

Monday, 18 September

08:45-09:00		Welcome: JL Puel, R Pujol Workshop opening: BM Johnstone
		Session I : *Neurotransmission* Moderators: M Eybalin, A Lysakowski
09:00 - 09:15	O1	Otoferlin as a hair-cell synaptic-complex protein Ramakrishnan NA, Drescher DG
09:15 - 09:30	O2	A Monte Carlo model of calcium dynamics in vertebrate hair cells: role of influx, buffering, diffusion and sequestration Bortolozzi M, Lelli A, Mammano F
09:30 - 09:45	O3	Persistence of $Ca_v1.3$ Ca^{2+} channels in mature outer hair cells supports afferent signalling of type II fibres Engel J, Knirsch K, Brandt N, Braig C, Schug N, Kuhn S, Münkner M, Knipper M
09:45 - 10:00	O4	Proteins associated with nicotinic receptor clustering in cochlear hair cells Osman AA, Schrader AD, Hawkes AJ, Simmons DD
10:00 - 10:15	O5	Ultrastructural and confocal localization of calcium store channels in cochlear and vestibular hair cells and ganglia Lysakowski A, Gaboyard S, Price SD, Klapczynski M, Cameron P
10:15 - 10:30	O6	Adrenergic Innervation of the organ of Corti Drescher MJ, Drescher DG, Ramakrishnan NA, Hatfield JS
10:30 - 11:00		Pause
		Session II : *Endolymph homeostasis* Moderators: S Bartolami, E Ferrary
11:00 - 11:15	O7	Distribution of TRPV4 in the human endolymphatic sac Kumagami H, Yamaguchi N, Fujiyama D, Baba A, Miyamoto I, Fukuda T, So K, Takasaki K, Takahashi H

11:15 - 11:30	O8	Regulation of the endocochlear potential by ATP Thorne PR, Chung M, Muñoz DJB, Wit HP, Housley GD
11:30 - 11:45	O9	Endocytosis of ion channels in the stria vascularis: Of any importance for hearing? Knipper M, Claussen C, Rüttiger L, Saftig P, Koenig O, Willnow TE, Gross M, Zimmermann U
11:45 - 12:00	O10	Expression of aquaporin-2, -3, -4, -6 and vasopressin type 2 receptor in the lateral wall of the cochlea Takeda T, Nishioka R, Kakigi A, Taguchi D
12:00 – 12:15	O11	In vivo visualization of endolymphatic hydrops in man Pyykkö I, Zou J, Daspidar P
12:15 – 12:30	O12	FXYD6, a novel cochlear regulator of Na,K-ATPase Delprat B, Schaer D, Roy S, Wang J, Renard N, Puel JL, Geering K
12:30 - 13:30		Lunch
13:30 - 14:30		**Poster session** (Presentation by authors of odd number posters only)
		Session III : *Rescue* Moderators: J Ashmore, J Ruel
14:30 - 14:45	O13	Animal models of salicylate and noise induced tinnitus and pharmacologic treatments Salvi R, Sun W, Lobarinas E, Yang G, Zhang L, Mirza N, Dalby Brown W
14:45 - 15:00	O14	Acoustic stimulation via open ear hearing aid: results in tinnitus therapy Del Bo L, Ambrosetti U, Fagnani E, Forti S, Scotti A
15:00 - 15:15	O15	Prevention of noise and drug-induced hearing loss with D-methionine Campbell K, Meech R, Rybak L, Klemens J, Hughes L
15:15 - 15:30	O16	Drug delivery to the inner ear through a port and septum attached to the CI housing and electrode: long term in vitro safety results Jolly C, Reetz G, Béal F, Baumgartner WD, Miller J

15:30 - 15:45	O17	Local administration of triamcinolone or dexamethasone preserve hearing after cochlear implantation in guinea pigs: CAP-measurements Braun S, Ye Q, Kiefer J, Gstöttner W, Tillein J
15:45 - 16:00	O18	TNFα is ototoxic to the hair cells of organ of Corti explants and dexamethasone protects these sensory receptor cells from damage by TNFα Van De Water T, Chen S, Eshraghi A, Jaben K, Haake S, Balkany T
16:00 - 16:30		Pause
		Session IV : *Ototoxicity and physiopathology* Moderators: J Schacht, J Wang
16:30 - 16:45	O19	Phosphoinositide signaling and cytoskeletal arrangements in the organ of Corti Sha SH, Jiang HY, Schacht J
16:45 - 17:00	O20	Vestibular toxicity of nitriles in the mouse and the guinea pig Díez-Padrisa N, Soler-Martín C, Boadas-Vaello P, Llorens J
17:00 - 17:15	O21	Thrombomodulin expression in the stria vascularis Sunami K, Kanazawa A, Okabe Y, Nishiura H, Takayama M, Inoue A, Yamane H
17:15 - 17:30	O22	The effects of several anaesthetics on auditory evoked compound action potentials and brainstem responses in the guinea pig Aarts MCJ, Verhaal J, Albers FWJ, Klis SFL
17:30 - 17:45	O23	Presbyacusis- a complex disorder with genetic and environmental background Toppila E, Manninen M, Zou J, Makela K, Varpa K, Pyykkö I
19:00		*Mayor Reception* (City Hall)*

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Tuesday, 19 September

Session V : *Mechanisms of development and repair*
Moderators: M Knipper, M Lenoir

09:00 - 09:15	O24	Brief noise exposure in juvenile rats causes a deterioration in the frequency selectivity of inferior colliculus neurons in adulthood Popelar J, Suta D, Syka J
09:15 - 09:30	O25	Expression of genes involved in the apoptosis in the organ of Corti, the modiolus and the stria vascularis of newborn rats Gross J, Mazurek B
09:30 - 09:45	O26	Sox 10 is not necessary for auditory neurons survival Breuskin I, Bodson M, Thelen N, Thiry M, Belachew S, Lefebvre PP, Malgrange B
09:45 - 10:00	O27	P2X2/3 receptor inhibition of BDNF-mediated spiral ganglion neurite outgrowth Housley GD, Greenwood D, Jagger DJ, Hoya N, Huang LC, Thorne PR, King BF, Wildman SS, Ryan AF
10:00 - 10:15	O28	Peripheral nerve regrowth in vivo through BDNF/ FGF treatment in deafened guinea pigs Glueckert R, Bitsche M, Miller J, Zhu Y, Prieskorn D, Altschuler R, Schrott-Fischer A
10:15 - 10:30	O29	Evidence for the involvement of JNK signaling in epithelial repair, proliferation and stereocilia development during hair cell regeneration Warchol ME, Speck JD
10:30 - 11:00		Pause

Session VI : *Deafness genes*
Moderators: K Avraham, D Hillaire

11:00 - 11:15	O30	Progression of the inner ear pathology of DDR1-null mice Meyer zum Gottesberge AM, Gohla A, Massing Th, Gross O, Vogel WF
11:15 - 11:30	O31	Hearing loss in mice with partially-deleted vitamin D receptor gene Zou J, Barth S, Minasyan A, Keisala T, Wang JH, Lou YR, Kalueff A, Pyykkö I, Tuohimaa P

11:30 - 11:45	O32	Novel pendrin and myosin VI mutations in humans and mice: Functional implications Avraham KB, Dror AA, Brownstein Z, Rzadzinska AK, Fuchs H, Hasson T, Steel KP, Hrabé de Angelis M
11:45 - 12:00	O33	Hearing impairment in an animal model of oculocutaneous albinism type I Murillo-Cuesta S, Cantero M, Rodríguez L, Lazcano J, Sánchez-Calderón H, Montoliu L, Varela-Nieto I
12:00 - 12:15	O34	Contribution of GJB6 to DFNB1 related hearing loss in Portuguese families Teixeira H, Fialho G, Caria H
12:15 - 12:30	O35	Catalase polymorphisms associated with noise-induced hearing loss in two independent noise-exposed populations Konings A, Van Laer L, Malczyk M, Carlsson PI, Bondeson ML, Fransen A, Borg E, Sliwinska-Kowalska M, Van Camp G
12:30 - 13:30		Lunch
13:30 - 14:30		**Poster session** (Presentation by authors of even number posters only)
		Session VII : *Micromechanics* Moderators: G Rebillard, J Santos-Sacchi
14:30 - 14:45	O36	The outer hair cell active force production in the cochlear environment Spector AA, Liao Z, Popel AS, Brownell WE
14:45 - 15:00	O37	Membrane tension effects on chloride binding to prestin Santos-Sacchi J, Song L
15:00 - 15:15	O38	Non-mammalian orthologs of prestin are electrogenic divalent/chloride exchangers Schaechinger TJ, Oliver D
15:15 - 15:30	O39	A low-coherence interferometry system and its use for vibration measurement and imaging of the guinea pig organ of Corti in vivo Chen F, Choudhury N, Zheng J, Matthews S, Jacques SL, Nuttall AL

15:30 - 15:45	O40	Group delay contour plots derived from human DPOAE level/phase maps Meinke DK, Lonsbury-Martin BL, Stagner BB, Martin GK
15:45 - 16:00	O41	Measuring olivocochlear feedback via modulation of otoacoustic emissions: An individualized measurement paradigm Wagner W, Heppelmann G, Müller J, Janssen T
16:00 - 16:15		**Spoendlin Junior Award** Moderators: JL Puel, R Pujol
16:15 - 16:30		Pause
16:30 – 17:30		IEB Business Meeting
19:30		*Gala Dinner** (Opera Comédie)*

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Wednesday, 20 September

Session VIII : *Cell and gene therapy*
Moderators: J Ito, F Venail

08:45 - 09:00	O42	Distribution of hematopoietic stem cell-derived cells in mouse cochlea and their possible roles Nakagawa T, Kita T, Okano T, Kada S, Kojima K, Ito J
09:00 - 09:15	O43	The role of bone marrow derived stem cells in inner ear repair Tan B, Ruan RS
09:15 - 09:30	O44	Cell transplantation to the auditory nerve and cochlear duct Kojima K, Sekiya T, Matsumoto M, Kim TS, Tamura T, Ito J
09:30 – 09:45	O45	BDNF gene delivery into the mouse cochlea by cell transplantation Okano T, Nakagawa T, Kita T, Ito J
09:45 - 10:00	O46	Time course of hair cell death in the cochlea of connexin30 knockout mice and its complete rescue by augmented connexin26 protein expression Ahmad S, Tang W, Chang Q, Qu Y, Hibshman J, Li YH, Söhl G, Willecke K, Chen P, Lin X
10:00 - 10:15	O47	Over-expression of XIAP delays the development of presbycusis in C57Bl/6 mice Wang J, Yu Z, Bance M, Morris D, Moore CS, Robertson GS, Korneluk R
10:15 - 10:45		Pause

Session IX : *Ionic signalling*
Moderators: C Chabbert, F Mammano

10:45 – 11:00	O48	The mechanotransducer channels of mammalian cochlear hair cells Fettiplace R, Beurg M, Hackney CM
11:00 - 11:15	O49	Local Ca^{2+} as indicator of the location of mechanoelectrical transduction channels Harasztosi C, Ramaswamy R, Müller B, Gummer AW
11:15 - 11:30	O50	Long-range signalling by calcium waves in the organ of Corti Mammano F, Anselmi F, Ciubotaru C, Gale JE, Piazza V

11:30 - 11:45	O51	Ultrastructural localization of large calcium activated potassium channels in the rat cochlea Mahendrasingam S, Furness DN, Fettiplace R, Hackney CM
11:45 - 12:00	O52	Acid sensing ionic channels in the vestibular endorgans Soto E, Mercado F, López I, Acuña D, Ortega A, Flores A, Vega R
12:00		End of the Meeting

IEB-2006 WORKSHOP

POSTERS

Posters will be posted from.Sunday 17th through Tuesday 19th at 18:00
Authors should be at their posters during poster presentation time (odd numbers on Monday 18, even numbers on Tuesday 19)

Normal structure and function

P1 Excitability properties of rat vestibulocerebellum interneurons
Bottà L, Masetto S, D'Angelo E

P2 Morphometry of the reticular lamina (SEM and LSM investigations on guinea pig cochlea)
Yarin Y, Meißner H, Bornitz M, Poznyakovskiy A, Zahnert Th

P3 A novel method for distinguishing between type I and type II spiral ganglion neuron input into the mouse cochlear nucleus
Barclay M, Huang LC, Hoya N, Ryan AF, Housley GD

P4 Two classes of outer hair cells along the tonotopic axis of the cochlea
Braig C, Engel J, Rüttiger L, Kuhn S, Münkner S, Hirt B, Rohbock K, Knipper M

Endolymph homeostasis

P5 Functional expressions of KCNQ1/KCNE1 K^+ channel and P2Y4 receptor
Lee JH, Hur DG, Heo JH, Chang SO, Kim CS, Oh SH

P6 Electrophysiological recordings of the utricular endolymphatic potassium concentration
Bartolami S, Zussy C, Quentin J, Desmadryl G, Chabbert C

P7 Expression of AQP2, vasopressin type 2 receptor, TRPV1 and TRPV4 in the human endolymphatic sac
Taguchi D, Kakigi A, Takeda T, Nishioka R, Takumida M, Kitano H

Micromechanics

P8 Prestin and a functional tectorial membrane are not required to generate electrically evoked otoacoustic emissions
Drexl M, Mellado Lagarde MM, Zuo J, Richardson GP, Russell IJ

P9 Basilar membrane response to electrical stimulation in mice with and without the tectorial membrane load
Mellado MM, Drexl M, Russell IJ

P10 Influence of the tectorial membrane resonance on the cochlear acoustical and neural responses
Lukashkin AN, Smith JK, Russell IJ

P11 The tectorial membrane: trading frequency selectivity and sensitivity in the mammalian cochlea
Russell IJ, Legan PK, Lukashkina VA, Lukashkin AN, Goodyear RJ, Richardson GP

P12 The effects of cholesterol on outer hair cell motility
Kitani R, Kakehata S, Murakoshi M, Wada H, Abe T, Namba A, Shinkawa H

P13 Electrogenic chloride and bicarbonate transport by prestin (SLC26A5) in expression systems
Sampedro-Castaneda M, Mistrik P, Ashmore JF

Ionic signalling

P14 Mechanisms of calcium homeostasis in cochlear hair cells
Nemzou R, Ohlrogge M, Cimica V, Tempel B, Moser T

P15 Calcium currents in mouse inner hair cells are inhibited by phenylalkylamines and benzothiazepines
Tarabova B, Lacinova L, Engel J

P16 BK channels regulation in rat inner hair cells
Hoang Dinh E, Dulon D

P17 Single channel pKir inwardly rectifying currents from pigeon vestibular hair cells
Zampini V, Masetto S, Correia MJ

Neurotransmission

P18 Temperature enhances exocytosis efficiency at the inner hair cell ribbon synapse
Nouvian R, Moser T

P19 Analysing the Ca^{2+} currents in inner and outer hair cells of mice lacking the β3- or β4 auxiliary Ca^{2+} channel β subunit
Kuhn S, Knirsch M, Rüttiger L, Kasperek S, Münkner S, Freichel M, Flockerzi V, Knipper M, Engel J

P20 Opioid receptor modulation of the calcium current in cochlear outer hair cells
Vega R, García-Garibay O, Soto E

P21 Fast and sustained exocytosis of inner hair cells co-vary with the synapse number along the tonotopic axis of the mouse cochlea
Meyer AC, Egner A, Yarin Y, Moser T

P22 Defective synaptic maturation in the inner hair cells of athyroid Pax8$^{-/-}$ mice
Sendin G, Bulankina A, Moser T

P23 Role of synaptic ribbons in hair cell sound coding
Buran BN, Khimich D, Gundelfinger E, Moser T, Liberman MC

P24 Glutamate increases cochlear dopaminergic neurotransmission through NMDA receptors and NO release
Halmos G, Horváth T, Polony G, Vizi ES, Lendvai B, Zelles T

P25 Maturation changes at the inner hair cell ribbon synapse
Eybalin M, Renard N, Schrott-Fischer A, Striessnig J, Puel JL

P26 Effects of human defensin NP-1 on synaptic transmission in the frog vestibular organs
Andrianov GN, Nozdrachev AD, Ryzhova IV

P27 Immunocytochemical and pharmacological studies of the metabotropic glutamate receptors in the frog semicircular canals
Andrianov GN, Puyal J, Raymond J, Ventéo S, Demêmes D, Ryzhova IV

P28 Evidence of functional purinergic receptors (P2X) in rat vestibular ganglion neurons
Ito K, Chihara Y, Sugasawa M, Sahara Y

P29 New insights in the vestibular calyceal neurotransmission
Bonsacquet J, Brugeaud A, Compan V, Desmadryl G, Chabbert C

Mechanisms of Development

P30 Notch signaling inhibitors increase hair cells in embryonic organ culture
Takebayashi S, Yabe D, Yamamoto N, Kojima K, Ito J, Honjo T

P31 mRNA expression of growth factors in the organ of Corti, modiolus and stria vascularis of newborn rats
Amarjargal N, Mazurek B, Machulik A, Fuchs FU, Haupt H, Ungethüm U, Kuban RJ, Gross J

P32 Developmental changes of cross-link arrangement and BAPTA sensitivity in rat cochlear hair bundles
Fink S, Koitschev A, Langer MG

P33 Age-related apoptotic change in the cochlear lateral wall of mice
Watanabe K, Inai S, Jinnouchi J, Baba S, Yagi T

P34 Developmental BK channel distribution in relation to the appearance of square arrays in the lateral membranes of mice inner hair cells
Mikiel-Hunter J, Forge A

P35 Estrogen receptors in the inner ear during different stages of pregnancy
Simonoska R, Stenberg E A, Sahlin L, Hultcrantz M

P36 Role of Sox10 in the development of the inner ear
Bodson M, Breuskin I, Thelen N, Belachew S, Thiry M, Lefebvre PP, Malgrange B

P37 Distribution of calbindin in the developing inner ear of the mouse
Buckiova D, Syka J

P38 Survival and differentiation of mouse embryonic statoacoustic ganglion cells in co-culture with auditory brain stem slices
Glavaski-Joksimovic A, Kostyszyn B, Eriksson M, Olivius P, Ulfendahl M

P39 Temporal and spatial regulation of α6 integrin expression during the development of the cochlear-vestibular ganglion
Davies D

Ototoxicity and physiopathology

P40 Cochlear effects induced by cisplatin and oxaliplatin – a dose escalation study
Hellberg V, Wallin I, Fransson A, Ehrsson H, Laurell G

P41 Microarray data of the newborn rat cochlea and their changes during cultivation and hypoxia
Haupt H, Mazurek B, Machulik A, Fuchs FU, Kuban RJ, Ungethüm U, Amarjargal N, Gross J

P42 Expression of hypoxia-dependent genes in the cochlea of the newborn rat
Mazurek B, Machulik A, Fuchs FU, Amarjargal N, Kuban RJ, Ungethüm U, Haupt H, Gross J

P43 Oxidative stress pathways in the potentiation of noise-induced hearing loss by acrylonitrile
Pouyatos B, Gearhart C, Nelson-Miller A, Fulton S, Fechter L

P44 siRNA-based elucidation of the impact of HIF-1α and HIF-2α on the expression of hypoxia-inducible genes in the organ of Corti of newborn rat
Henke W, Fuchs J, Mazurek B, Gross J

P45 Expression of the BK channel is severely delayed in IHCs of hypothyroid rats and shows a mosaic expression pattern
Brandt N, Münkner S, Braig C, Winter H, Knipper M, Engel J

P46 A mouse model for selective degeneration of the spiral ligament due to local application of 3-nitropropionic acid
Kada S, Nakagawa T, Ito J

P47 Differential gene expression of artemin, GDNF, BDNF and TGFβ in deafened rats following electrical stimulation
Wissel K, Prieskorn DM, Miller JM, Lenarz T, Stöver T

P48 Cisplatin-induced gene expression in the rat cochlea
Kirkegaard M, Skjönsberg Å, Bucinskaite V, Laurell G, Ulfendahl M

P49 Relationship between noise-induced hearing loss, enhanced amplitudes of cortical evoked responses and gap detection threshold changes
Rybalko N, Grecova J, Popelar J, Syka J

P50 Macrophages invasion in the amikacin treated rat cochlea
Ladrech S, Wang J, Simonneau L, Gallego M, Puel JL, Lenoir M

P51 Noise induced hearing loss (NIHL) and vestibular functional damage
Sergi B, Fetoni AR, Paludetti G, Rizzo D, La Greca C, Troiani D, Ferraresi A

P52 The vestibular toxicity of cis-crotononitrile does not require CYP2E1-mediated metabolism
Boadas-Vaello P, Díez-Padrisa N, Llorens J

P53 Identification of markers for neuronal plasticity in the auditory system
Singer W, Panford-Walsh R, Haas H, Rüttiger L, Köpschall I, Rohbock K, Zimmermann U, Knipper M

P54 No difference between inner and outer hair cells in uptake of FM1-43
Taylor R, Forge A

P55 Up-regulation of Notch signaling following ototoxic deafening
Batts SA, Shoemaker CR, Raphael Y

P56 Middle ear gas loss in inflammatory conditions: The role of mucosa thickness and blood flow
Amos AR, Herman P, Lecain E, Wassef M, Tran Ba Huy P, Kania RE

P57 Cubilin and megalin co-localize in the inner ear
Tauris J, Christensen EI, Nykjaer A, Jacobsen C, Ovesen T

P58 Neuronal activity in the inferior colliculus and auditory cortex in rats with noise-induced altered gap detection ability
Profant O, Burianová J, Popelar J, Syka J

P59 Meniere's disease and the electromodel
Offutt G

Gene

P60 Association of SNPs in KCNE1 and KCNE3 genes with Meniere's disease
Doi K, Sato T, Kuramasu T, Nishimura M, Kubo T

P61 KCNQ4, a gene for age related hearing impairment
Van Eyken E, Van Laer L, Fransen E, Topsakal V, Lemkens N, Laureys W, Nelissen N, Vandevelde A, Wienker T, Van De Heyning P, Van Camp G

P62 Auditory neuropathy in a hereditary mitochondrial disease : Evaluation of hearing function in Friedreich ataxia mouse transgenic model
Giraudet F, Martelli A, Puccio H, Mom T, Avan P

Protection, repair and regeneration

P63 Protective effect of Ebselen on noise induced cochlear damage in rat
Park YH, Kim Y

P64 TEM analysis of the synaptic consequences of hair cell loss and regeneration in a bird with progressive, genetic inner ear abnormality
Ryals BM, Sanovich E, Petralia RS, Dooling RJ

P65 Protective properties of Idebenone and Vitamin E in noise induced hearing loss in the guinea pigs
Fetoni AR, Sergi B, La Greca C, Rizzo D, Troiani D, Ferraresi A, Paludetti G

P66 Pharmacological protection against cisplatin induced ototoxicity
Lorito G, Giordano P, Sathiyaseelan T, Petruccelli J, Magosso S, Bertolaso L, Martini A, Hatzopoulos S

P67 Ethanolic extracts from *Hemidesmus Indicus Linn.* displays otoprotectant activities on organotypic cultures
Corbacella E, Bertolaso L, Previati M, Martini A

P68 Quantitative analysis of surviving spiral ganglion cells in BDNF-treated cochleas of deafened guinea pigs
Agterberg MJH, De Groot JCMJ, Versnel H, Albers FWJ, Klis SFL

P69 Effects of combined electrical stimulation and GDNF treatment on spiral ganglion cells in deafened guinea pigs
Scheper V, Paasche G, Lenarz T, Stöver T

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SYMPOSIUM

Hearing Rehabilitation and Innovative Inner Ear Therapy

Abstracts

Session I: *Hearing Rehabilitation*

S1

Hearing aids and brain plasticity

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Cerebral functional plasticity under auditory deprivation has been well established in animals and many recent works describe it in humans also. The observation that injury-related plasticity results in broad changes in cortical sensory maps, with over-representation of certain regions of the receptor surface, leads to the hypothesis that cochlear lesions might induce frequency-specific improvements in auditory discrimination performance. On the other hand, there have not been many studies showing plasticity under rehabilitation related to hearing aid fitting. However, tonotopic map reorganisation following auditory rehabilitation is now being suggested on the basis of the sole criterion of frequency discrimination at the cut-off frequency. Likewise, various studies have highlighted improvements in sound performance, intensity discrimination, temporal discrimination and auditory lateralisation following hearing aid fitting.
This presentation will give a synthetic review of the recent studies underlying these conclusions in favor of a cerebral auditory reorganization following deprivation and rehabilitation.

S2

New developments in hearing aid technology

Chalupper S

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Digital signal processing allows to implement more and more sophisticated techniques to improve the benefit of hearing aids for hearing impaired listeners. Two recently introduced developments are 1) trainable hearing aids and 2) an algorithm (“SoundSmoothing”) which reduces the annoyance of impulsive, transient noises. Trainable heaing aids aim at automatically optimizing the hearing aid setting according to individual and situation specific preferences – without the need of visiting an audiologist. SoundSmoothing is designed to reduce the annoyance of transient noise in order to support the acclimatization to hearing aid amplification. The functional principles of both algorithms will be explained and results of clinical studies will be presented.

S3

Implantable middle ear devices

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30% of the population over 65 years of age is hearing impaired, corresponding to 7% of the general population. At the present time, this frequent handicap can only reduced by the use of hearing aids allowing todeliver higher sound energy to the inner ear. These prosthesis have undergone tremendous improvement over the last few years in particular on the electronic and aesthetic aspects. In this presentation, we will review the progresses which have been made on implantable hearing devices transmitting the sound energy directly to the ossicular chain in the middle ear.

Semi implantable devices are composed of an external part containing the microphone, the battery and the electronic transferring the information transcutaneously to the internal receiver which activates the transducer attached to the ossicular chain.

In the fully Implantable Hearing Device from Otologics Inc., the subcutaneous microphone picks up ambient sounds, converts them into an electrical signal, amplifies the signal according to the wearer's needs, and sends it to an electro-mechanical transducer. The transducer tip is mounted in a laser-drilled hole in the body of the incus and translates the electrical signal into a mechanical motion that directly stimulates the ossicles and enables the wearer to perceive sound. The implanted battery is recharged daily via an external charger and the wearer can turn the implant on and off with a hand held remote control.

S4

New trends in cochlear implantation

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Over the last 20 years, cochlear implantation has become a widely accepted method of managing profoundly deaf children and adults. Results have been impressive and indications for a cochlear implant have been extended continuously. The manufacturers of the devices have made great efforts to miniaturize the external and internal components of the system, and new developments in electrode arrays, combined with new speech coding strategies have resulted in considerable improvements in the outcomes.
This presentation will focus on new trends and future development of cochlear implantation: possibility of preservation of low-frequency hearing using atraumatic cochlear implant electrode, insertion procedures for combined, ipsilateral electric and acoustic stimulation, brainstem implantations.

Session II: *Pharmacology*

S5

From brain molecular pharmacology to clinical applications

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Our understanding of the brain function began with the discovery of the neurons, their electrical activity and the identification of the chemical processes allowing neuronal communication. First identified at the neuromuscular junction, nicotinic acetylcholine receptors have for a long time been used as a model of ligand-gated ion channels that mediate neurotransmission. With the cloning and sequencing of the muscular nicotinic acetylcholine receptors it became possible to examine related proteins and it was discovered that an entire family of genes encode for nicotinic acetylcholine receptors that are widely expressed in neuronal and non-neuronal cells. The natural alkaloid nicotine contained in tobacco leaves that was used since the early days of pharmacology to distinguish between two types of neurotransmission mediated on one hand by muscarine sensitive receptors and on the other hand by nicotine sensitive receptors. These steps resolved numerous issues about the effects of chemical substances on the nervous system. While progresses were quickly realized at ganglionic and central nicotinic acetylcholine receptors a shadow remained on the chemical transmission of the inner ear with the outer hair cells showing an ambiguous muscarinic and nicotinic pharmacological profile. The identification and cloning of two genes encoding for the α9 and α10 subunits of the nicotinic acetylcholine receptor family revealed unique properties that account for the pharmacological profile of the inner ear and vestibule. Sequencing of the entire human genome and first studies of single nucleotide polymorphisms (SNPs) marked a new and important step in our understanding of common traits and variants that are our individual characteristics. Functional studies allow the characterization of these genetic polymorphisms on receptor functions and it becomes possible to correlate such genetic variation to physiological differences.

S6

Local therapy of hearing loss and tinnitus

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Recent advances in molecular pharmacology of the cochlea have lead to a much better understanding of the physiology, and especially the physiopathology, of the sensorineural structures of the organ of Corti. Knowledge of the intimate molecular mechanisms of cellular dysfunction is of considerable use in the development of new therapeutic strategies. We will summarize the mechanisms of sensory hair cell death after various injuries. Among these strategies, the use of D-JNKI-1, a cell permeable JNK-ligand that blocks the activation of c-Jun is very promising to prevent impairment due to noise or ototoxic drugs. Actually, round window delivery of D-JNKI-1 prevents both hair cell death and development of a permanent hearing loss induced by acoustic trauma and neomycin. Furthermore, round window delivery of D-JNKI-1 after acoustic trauma can protect the cochlea if given within a therapeutic window of 12 hours. In addition to permanent hearing loss, exposure to noise or ototoxic drugs also induces tinnitus. We thus review recent findings obtained from a behavioural model of tinnitus in rats. In addition to providing evidence for the site and mechanism of generation of tinnitus induced by salicylate, these results support the idea that targeting cochlear N-methyl-D-aspartate receptors may represent a promising therapeutic strategy for treating tinnitus. Our approaches will be discussed in terms of conservation of residual hearing and/or treatment of tinnitus occurring during and following cochlear implantation.

S7

Mechanisms of auditory hair cell death in response to oxidative stress initiated damage and otoprotective strategies to preserve hearing

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Oxidative stress is generated within the cochlea in response to a variety of insults, e.g. exposure to ototoxins, sound trauma and physical trauma. Oxidative stress damages cochlear hair cells and one way in which a damaged hair cell may die is via apoptosis. Apoptosis is an active process and requires the participation of a cell death signal cascade. One such signal cascade demonstrated to participate in the apoptotic cell death of damaged hair cells is the mitogen activated protein kinase (MAPK)/ c-Jun-N-terminal kinase (JNK) pathway. The MAPK/JNK signal cascade has been implicated in the apoptosis of damaged hair cells in response to neomycin ototoxicity, sound trauma, and electrode insertional trauma (EIT) generated hearing loss. D-JNKI-1 peptide is a binding protein for all 3 isoforms of JNK and acts through competitive binding.
D-JNKI-1 peptide was tested in an animal model of EIT-initiated hearing loss and was shown to provide almost complete protection against the loss of hearing threshold that occurred post-EIT. Because D-JNKI-1 has its inhibitory action through direct binding of JNK molecules this result demonstrates that the MAPK/JNK signal cascade participates in the hearing impairment caused by EIT. Another series of experiments investigated the otoprotective capacity of a corticosteroid, i.e. dexamethasone (DXM), in our model of cochlear implantation trauma, i.e. EIT. In these EIT experiments DXM delivered locally into the cochlear was found to be almost as effective as D-JNKI-1 in preventing EIT-initiated hearing loss. DXM is both a powerful inhibitor of the inflammatory process and demonstrated to be an inhibitor of JNK signaling.
These results implicate both MAPK/JNK signaling and the inflammatory process in EIT-initiated hearing loss. Blocking these cellular events may therefore be an effective complement to cochlear implantation when conservation of residual hearing is an important factor.
Supported by MED-EL & Advanced Bionics Corporation.

S8

Gene Expression in Cisplatin Ototoxicity and Protection with p53 Inhibitor

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Cisplatin damages the hair cells and supporting cells in the inner ear through cell death signaling pathways that are not fully understood. We used focused apoptosis gene microarrays to study the early changes in gene expression in cochlear cultures treated with cisplatin (0. 2 mM). After 12 h of cisplatin treatment more than 50% of the 96 genes on the array showed a significant decrease in expression, consistent with the fact that many cells were in the process of dying at this time. However, after 3 h of cisplatin treatment, during the early stage of cisplatin-induced stress, 5 genes showed significantly increases in expression. Gadd45, an inducible protein regulated by p53, was upregulated; this gene has been shown to be upregulated in other systems by cisplatin. Mydd88, myeloid differentiation primary response gene, a member of the death domain family, shows coordinated expression with Gadd45. Tnfaip2 (B94), a tumor necrosis factor alpha induced protein, has been shown to be upregulated in coronary artery disease and Alzheimer disease. Mcl-1, a Bcl-2 family protein, promotes cell survival by interfering with early events associated with the release of cytochrome c from mitochondria. Tnfrsf1a, a member of the tumor necrosis factor receptor superfamily, encodes a protein that is a major receptor for tumor necrosis factor-alpha; this receptor activates NF-kB, mediates apoptosis, and regulates inflammation. Since p53 regulates Gadd45, p53 immunolabeling and Western blots were carried out after cisplatin treatment. Cisplatin induced expression of phosphor-p53 serine 15 immunolabeling in cochlear culture; p53 expression in Western blots was first observed at 6 h post-treatment and increased in expression over the next 48 h. Pifithrin-alpha, a p53 inhibitor, blocked the express of p53 and caspase 3, and protected against cisplatin induced hair cell loss in a dose-dependent manner. Supported by NIH grant R01 DC00630

Session III: *Cell and gene therapy*

S9

Cellular therapy for inner ear disorders

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Placement of cells into the inner ear offers multiple opportunities for treating hearing disorders. Research on cellular therapy has illuminated both the potential and the limitations of this approach.
Pharmacotherapy of the inner ear requires methods for the delivery of bioactive compounds. Utilization of cells as biological "factories" for compounds is an attractive alternative to pumps or middle ear delivery, with the potential for stable, long-term delivery. Potential uses include the delivery of factors that might support the survival of hair cells or neurons in the inner ear. For example, cells transformed to produce neurotrophins could replace trophic support that is lost when hair cells are absent. Similarly, transformed cells produced tropic and other guidance factors could be use to guide the regrowth of spiral ganglion dendrites toward a cochlear implant.
The transplantation of cells to replace lost or damaged tissue is another possibility that has received wide attention. Stem cells can, in theory, adopt the phenotype of any cell that is required. Transplantation of many types of stem cells into the inner ear has proven to be relatively straightforward. Integration of cells into the appropriate locations and adoption a desired cellular phenotype has been much more difficult to achieve, but progress has been made. Transplantation of immature hair cells into damaged sensory epithelia has been accomplished, and is accompanied by integration and regrowth of stereocilia. Successful transplantation is limited to a critical period of hair cell development, however. Moreover, access to the epithelia that would allow transplantation in vivo remains a problem.
The use of autologous cells for transfer would avoid issues of rejection. Fortunately, several sources of autologous cells might be suitable for inner ear implantation, including stem cells and cells that could be used for production of biomolecules.

(Supported by the NIH/NIDCD and the VA.)

S10

Strategies to regenerate hair cells: identification and differentiation of progenitors

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Perception deafness, as opposed to transmission deafness, results from a lesion of the sensory cells and/or of the neurons of the auditory part of inner ear. There is currently no treatment able to stop the progression of a hearing loss or to restore a lost auditory function. In designing therapy for sensorineural deafness, the most important task is to find a way to generate new cochlear hair cells to replace lost cells. A spontaneous post-lesional regeneration of hair cells in the sensory epithelium has been observed in amphibians and birds as well as in the vestibular part of the mammalian inner ear. In contrast, in mammalian cochlea, hair cells are believed to be produced only during embryogenesis; after maturity, sensory or supporting cell proliferation or regeneration are thought to occur neither under normal conditions nor after trauma.
We described here that progenitor cells, isolated from newborn rats, proliferate and differentiate in hair cells and supporting cells. Furthermore, we showed that supernumerary hair cells arise in the cultured organ of Corti and can be recruited in the presence of cell cycle regulators. The persistence of sensory cell progenitors in adult mammalian organ of Corti and the understanding of the mechanisms leading to the production of hair cells, in the developing cochlea, open prospects of hair cell regeneration in the mature inner ear.

S11

Developing stem cell-based therapies for deafness: Isolation and differentiation of human fetal auditory stem Cells (hFASCs)

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Hearing impairment is an irreversible condition caused by the loss of sensory hair cells and neurons. The lack of regenerative potential of the human cochlea is due to the fact that progenitors are only produced transiently during embryonic development. There is no cure for deafness although, if neurons are preserved, the sensory function of the ear can be replaced by a cochlear implant.
A potential therapeutic approach could involve the transplantation of appropriate stem cells. Such approach may also provide a mean of delivering neurotrophins to promote the survival of neurons, improving the performance of implanted patients.
Due to the lack of regenerative response in the adult cochlea, we have used the foetal auditory organ as a source for stem cell isolation. By carefully microdisecting the sensory epithelia from 9-11 weeks-old fetuses and using optimized culture conditions including different growth factors we have selectivetly expanded a population of cells that expressed NESTIN, SOX2 and other markers normally associated with the stem cell phenotype. After several months of passaging in vitro, cells remain proliferative and undifferentiated. We have defined culture conditions that induce differentiation into hair cells and neurons. When transferred to neuralizing conditions, cells extend processes and readily differentiate into bipolar auditory neurons that express neurogenin, brn3a, beta-tubulin III and neurofilaments. They also display typical neuronal potassium and sodium currents. When exposed to hair cell conditions, several hair cell markers as well as potassium and calcium currents are induced.
Experimental work with the human cochlea has been limited to clinical measurements and the analysis of surgical or post-mortem samples. A human in vitro system such as this does not only offers a potential therapeutic application, but it could become a useful model for experimentation and drug testing.

S12

Probing the structure and function of the tectorial membrane in tectorin mutant mice

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The mammalian tectorial contains three collagens and three non-collagenous proteins, Tecta, Tectb and otogelin - glycoproteins that are only expressed at high levels in the inner ear. In humans, mutations in TECTA cause hereditary deafnesses with a variety of clinical phenotypes. To determine how the tectorins contribute to tectorial membrane matrix structure, and to help understand how the tectorial membrane influences cochlear function, we have made a series of transgenic mice with mutations in Tecta and Tectb.

Mice homozygous for a large deletion ($Tecta^{\Delta ENT/\Delta ENT}$) in Tecta have tectorial membranes that lack all non-collagenous matrix structures and are completely detached from the organ of Corti. These mice reveal the tectorial membrane plays a key role in ensuring outer hair cells are displacement coupled and therefore able to deliver feedback with optimal gain and timing.

Mice heterozygous for the missense mutation ($Tecta^{+/Y1870C}$) causing deafness in the Austrian DFNA8/12 family have attached tectorial membranes with a much-reduced limbal attachment zone and a greatly enlarged subtectorial space in the vicinity of the inner hair cells. Tuning and sensitivity of the basilar membrane is little affected, but a sharp loss in sensitivity seen at the tips of the neural tuning curves indicates the tectorial membrane plays an essential role in driving the inner hair cells at their best frequency.

Mice homozygous for a large deletion of the Tectb gene ($Tectb^{-/-}$) have attached tectorial membranes, but the matrix within which the radial collagen fibrils are usually imbedded lacks organisation, and the cross-sectional area of the tectorial membrane is greatly enlarged at the apical end of the cochlea. Although these mice have a low-frequency hearing loss, at high frequencies (>20 kHz) both basilar membrane and neural tuning curves are considerably sharper than those of wild type mice, possibly due to a reduction in longitudinal coupling within the tectorial membrane.

Session IV: *Cell and gene therapy (continued)*

S13

Re-activation of developmental signaling for repair of mature inner ears

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Auditory hair cell loss in mammals is permanent although other vertebrates spontaneously regenerate hair cells. Hair cell regeneration in birds and other non-mammalian vertebrates is accomplished through transdifferentiation of non-sensory cells, with or without cell division. Experimentally forced expression of developmental genes aimed at reactivating developmental programs in the mature ear can induce transdifferentiation in the mammalian cochlea. We have been refining and extending the strategies for inducing hair cell regeneration in the mature organ of Corti. We have determined that the morphology of remaining non-sensory cells in the deaf ear is important for a successful outcome of developmental program reactivation. Specifically, a simple epithelial layer of cells in the organ of Corti, which can present itself after a severe injury, does not respond to *Atoh1* expression therapy. We have also found that some of the genes that regulate initial hair cell development, specifically those in the Notch signaling cascade, alter their expression after a lesion is produced in the mature organ of Corti. Changes in the expression of Notch signaling molecules may present targets for inducing phenotypic conversion of non-sensory cells to new hair cells. In another set of experiments, we forced the expression of a proliferation signal, *SKP2*, and a pro-hair cell developmental signal, *Atoh1*, in the mature ear, and determined that over-expression of *SKP2* can increase the number of ectopic hair cells generated by *Atoh1*. To advance hair cell regeneration therapy towards clinical application, it is necessary to further experiment with combinations of genes to create an optimal match for the condition of the recipient tissue.

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S14

Human hereditary deafness: from genes to the underlying pathogenic processes

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More than a hundred genes are predicted to underlie isolated forms of hereditary sensorineural deafness in humans. To date, 42 of them have been identified. The pathogenic processes involved in each of these forms of deafness are progressively deciphered.
Among the hereditary sensorineural forms, auditory neuropathies form a rare class that can be distinguished on clinical bases, mainly the persistence of otoacoustic emissions. So far, the causative genes have been isolated only for two of them, DFNB9 and DFNB59, which involve two novel proteins, otoferlin, a member of the ferlin protein family and pejvakin which shares sequence similarity with the gene defective in another form of deafness, DFNA5. The study of the mouse models generated for each of them has revealed that DFNB9 is an inner hair cell synaptopathy whereas DFNB59 is a neuronopathy affecting the auditory pathway downstream from the cochlea. Consistently, DFNB9-affected patients benefit from cochlear implants. This illustrates the interest of distinguishing between the diverse pathophysiological mechanisms underlying auditory neuropathies in a therapeutic perspective.

Closing Lecture

S15

The new era of hearing habilitation

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This presentation will consider the major sources of sensorineural hearing loss, what may be future modes of biologic treatment through regenerative medicine and pharmaceutical development, and where the field of auditory science is with respect to such treatments. I will focus on new strategies that have emerged in the past two decades and apparent opportunities for the future. Recent studies from University of Washington laboratories using the zebrafish lateral line neuromasts to screen for endogenous and exogenous molecules that influence the susceptibility of hair cells to ototoxic agents will be presented as one such approach. Attempts to validate this model and the development of screening procedures will be described. Using this approach we have discovered several new genes and pharmaceutical agents that modulate hair cell susceptibility to aminoglycoside ototoxicity. I will describe a few of these studies and then consider the strengths and weaknesses of this approach.

IEB-2006 WORKSHOP

Oral Presentations

Abstracts

Session I: *Neurotransmission*

O1

Otoferlin as a hair-cell synaptic-complex protein

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Mechanoreceptive hair cells lack synaptotagmin, and express the L-type calcium channel, Cav1.3. In the present work, we examined the interaction of mouse otoferlin C2 domains with the Cav1.3 II-III loop. Otoferlin, a protein which, in mutated form, causes non-syndromic deafness DFNB9 in humans, has molecular features of a calcium sensor and may substitute for synaptotagmin at the hair-cell synaptic complex. Otoferlin contains six C2, phospholipid/protein-interacting domains and a C-terminal transmembrane region. We cloned the six C2 domains (C2A-F) in prey vector pGADT7 and determined their interaction with the Cav1.3 II-III loop, present in bait construct pGBKT7. Both constructs were used in co-transformation experiments in yeast strain AH109, and the transformed cells were stringently screened with a quadruple drop-out medium (SD/-His/-Lys/-Trp/-Ade). After two days of incubation at 30 degrees C, colonies appeared for the C2D + II-III loop combination. The colonies reached >2 mm diameter and were white in color, indicating true interaction. After four days of incubation, a few small colonies also developed for the C2A and C2B domains. Reversal of bait and prey constructs in the protocol resulted in similar results, with robust colonies developing within two days for the C2D domain, but only a few, small colonies appearing for the C2B domain after four days and no colonies developing for the C2A domain after five days. In further experiments, we created Cav1.3 II-III loop mutants to identify specific loci within the II-III loop that may mediate C2D interaction. Deletion mutants in which putative SH3 or PDZ III binding motifs were removed showed very little interaction in yeast two-hybrid assays, whereas deletion in other regions of the II-III loop did not affect interaction. These results suggest that the Cav1.3 calcium channel and otoferlin may interact at the hair-cell synaptic complex as part of the calcium-sensing mechanism of exocytosis.

O2

A Monte Carlo model of calcium dynamics in vertebrate hair cells: role of influx, buffering, diffusion and sequestration

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In the vertebrate inner ear, sensory hair cells synapse onto afferent terminal of the acoustic or vestibular nerve. Hair cell synapses are capable of coding acoustic and vestibular information over a wide intensity range with high temporal precision and over prolonged periods of time. This ability depends largely on the mechanisms employed by the hair cell for rapidly modifying the cytosolic Ca^{2+} concentration ($[Ca^{2+}]i$) in the vicinity of transmitter release sites, where exocytosis might be predominantly controlled by one or few Ca^{2+} channels located in nanometer proximity from the release site of a vesicle. The study of $[Ca^{2+}]i$ dynamics in living cells typically combines Ca^{2+}-sensitive fluorescent indicators, patch clamp and optical microscopy to produce images of the patterns of fluorescence of a Ca^{2+} indicator (complexed to Ca^{2+}) following various stimulation protocols. To extrapolate $[Ca^{2+}]i$ dynamics from fluorescence imaging data we developed a simulation code based on the Monte Carlo method. We simulated the entry of Ca^{2+}, following cell depolarization, at individual presynaptic active zones (hotspots) over ~1 s time intervals and distances in the range from ~100 nm to the entire cell length (20-30 µm). The realistic reconstruction, in three dimensions, of the cellular boundaries and the computation of a virtual fluorescence ratio $\Delta f/f0$ equivalent to the one obtained from fluorescence microscopy experiments allowed us (i) to directly compare simulations to experimental data; (ii) to supply an estimate of the equivalent concentration and kinetics of Ca^{2+} reactants (buffers); (iii) to investigate the role of Ca^{2+}-ATPases (PMCA and SERCA pumps) during and immediately after Ca^{2+} influx through voltage-gated channels. We demonstrated that the mass action law hypothesis, used in the derivation of $[Ca^{2+}]i$ from fluorescence data, brakes down whenever system's equilibrium is perturbed, e.g. during Ca^{2+} influx.

O3

Persistence of $Ca_v1.3$ Ca^{2+} channels in mature outer hair cells supports afferent signalling of type II fibres

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Outer hair cells (OHCs) are innervated by type II afferent fibres of as yet unknown function. If these fibres indeed relayed afferent information from the OHCs, presynaptic Ca^{2+} channels would be required. By using the whole cell patch clamp technique we recorded Ca^{2+} channel currents with 10 mM Ba^{2+} as a charge carrier in OHCs and IHCs of mice and rats between P1 and P32. L-type Ca^{2+} channel currents showed developmental peak values of 165 pA (mouse) and 188 pA (rat) at P2, respectively. Currents declined upon OHC maturation in parallel to the acquisition of a mature OHC phenotype. Mouse OHC IBa decreased to <20 pA at P19 whereas average IBa in the more robust rat OHCs amounted to 66 ± 18 pA (n=13; P28). Properties of rat IHC and OHC currents were similar to those of neonatal mouse IHC/OHC Ba^{2+} currents carried by the Cav1.3 subunit (Platzer et al. Cell 2000; Michna et al. J. Physiol. 2003). Whole-mount in situ hybridisation, RT-PCR from selected IHCs and OHCs and immunostaining confirmed the presence of Cav1.3 in mature IHCs and OHCs. Mature rat OHCs showed reduced Ba^{2+} currents (30 %) compared to IHCs (219 ± 26 pA, n=6, P30). Assuming that rat OHCs are innervated by about 3 type II afferent fibers and IHCs by about 12 type I afferent fibers, the amount of IBa per afferent fiber would be of the same size in OHCs and IHCs (22 pA vs. 18 pA, respectively) which suggests that OHCs are capable of exocytosis. The presence of the synaptic vesicle protein otoferlin and the presynaptic protein CtBP2 add further pieces of evidence for functional exocytosis in OHCs.

O4

Proteins associated with nicotinic receptor clustering in cochlear hair cells

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Nicotinic acetylcholine receptors (nAChRs) mediate synaptic transmission between hair cells and olivocochlear (OC) axons in the mammalian cochlea. During development, OC axons form transient connections with inner hair cells (IHCs) prior to making permanent connections with outer hair cells (OHCs). Recent evidence suggests that IHCs are also capable of postsynaptic acetylcholine responses and may be clustered at synaptic junctions. At the neuromuscular junction (NMJ), several proteins, including muscle-specific kinase (MuSK) and rapsyn, are essential for clustering and stabilizing nAChRs. Previously we have shown that clusters of nAChRs can be visualized using α-bungarotoxin (α-BTX) labeling in vitro. At E18, there are no visible α-BTX plaques in the organ of Corti. At P0, α-BTX plaques are almost exclusively observed on IHCs in middle basal regions of the cochlea. By P10, α-BTX -labeled plaques on both IHCs and OHCs co-localize with OC terminals.

In the present study, we characterize the role of rapsyn and MuSK in the inner ear. First, we examined MuSK and rapsyn expression in the developing rodent cochlea. Our RT-PCR data suggest that rapsyn expression is relatively constant during the first two weeks of postnatal development. In contrast, MuSK expression peaks after the first week of postnatal development and then persists at lower levels. Immunofluorescent studies show rapsyn and MuSK localized to the hair cells. Second, we examined whether rapsyn interacts with the nAChR cytoplasmic loop. Interaction between α9 and rapsyn was demonstrated by co-immunoprecipitation both in cotransfected HEK293 cells and in cochlear homogenates. Third, we examined the effect of rapsyn on α-BTX labeling of α9 and α10 nAChRs in HEK293 cells. Expression of rapsyn with α9 causes an increase in α-BTX labeling compared with α9 alone. Thus, our data support the view that a clustering scaffold similar to the NMJ model may exist in the cochlea.

O5

Ultrastructural and confocal localization of calcium store channels in cochlear and vestibular hair cells and ganglia

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Ryanodine receptor (RyR) and inositol triphosphate (IP3) calcium store channels have been shown to be widely expressed in a variety of mammalian tissues. There have been few studies, however, on their localization in the vestibular periphery and cochlea. A functional model, put forth by Sridhar et al. (1997) for cochlea, where outer hair cells are contacted by efferent boutons opposite subsynaptic cisterns, is that the cistern creates a microdomain for a-9 nicotonic receptors to interact with calcium-gated potassium channels. They gave evidence for RyR involvement in this process. Other studies have provided functional evidence for IP3 involvement.
We performed an initial screen with RT-PCR and found all six isoforms (RyR1, RyR2, RyR3, IP3R1, IP3R2, IP3R3) present in the vestibular and cochlear endorgans and ganglia (Cameron et al., ARO, 2004). In the present study, immunogold EM and confocal microscopy were used to ascertain which RyR and IP3R isoforms were expressed specifically within hair cells in the cristae, maculae, cochlea, and vestibular and spiral ganglia. Western blots and preabsorption controls are used to confirm specificity of the antibodies.
With confocal microscopy, we have so far found IP3R1 to be a candidate for the subsynaptic cisternal isoform in outer hair cells (OHCs). In the crista and macula, IP3R1 is also found in type II hair cells and in calyces from the central/striolar zone. Confocal and electron microscopy confirmed the presence of RyR3 in Deiters cells, in inner hair cells (IHCs), and in some of their afferent endings, but not in OHCs, although RyR3 is present in efferent boutons on OHCs. All 6 isoforms are found in ganglion cells. In summary, specific isoforms of RyR and IP3 are both present in hair cells and ganglia in the rat inner ear, and may play a specific role in efferent neurotransmission.

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O6

Adrenergic innervation of the organ of corti

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The possibility that the organ of Corti (OC) directly receives adrenergic innervation is supported by the finding that dopamine beta-hydroxylase (DBH), the enzyme of synthesis of norepinephrine and an adrenergic marker, is expressed within the OC (Drescher et al., Neuroscience, 2006, in press). Further, alpha 1 (A1), beta 1 (B1) and beta 2 (B2) adrenergic receptors (AR) have been immunolocalized to sites within the OC well beyond the habenula perforata (Khan et al., ARO Abstr. 29: 300, 2006). To identify neural sites of expression and pre- and/or post-synaptic localizations, we have now compared immunofluorescence (IF) localization of A1, B1 and B2 ARs with IF for the adrenergic neuronal marker DBH and that for the efferent neuronal marker choline acetyltransferase (ChAT). Immunofluorescence for A1 AR was localized to apical sites on the inner hair cell and outer hair cells (OHC) in the apical and upper middle turns of the rat cochlea, appearing to overlap DBH reactivity, suggesting presynaptic localization in adrenergic fibers. B1 and B2 AR IF was found at apical sites on the OHC continuing down the length of the OHC in what appeared to be small-caliber nerve fibers, similarly to DBH IF. However, in addition, a possible post-synaptic localization on OHC was inferred for B1 at apical sites, with B1 IF extending beyond overlapping DBH-containing nerve fibers, cut in cross section. Immunofluorescence for the ARs was observed in small nerve fibers at the base of the OHC, adjacent to ChAT-containing nerve fibers which themselves were not reactive for the ARs. Immunofluorescence for serotonin, previously noted to be similar in its immunolocalization to norepinephrine (Drescher et al., ARO Abstr. 26: 12, 2003), appeared to overlap DBH but not ChAT IF within the OC. Sympathetic nerve fibers entering with blood vessels in the spiral limbus may constitute a source of adrenergic innervation for the OC/spiral ganglion, based on IF nerve traces for the ARs and DBH.

Session II: *Endolymph homeostasis*

O7

Distribution of TRPV 4 in the human endolymphatic sac

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Transient receptor potential vanilloid (TRPV) channels are calcium-permeable, nonselective cationic channels having a mechanosensitive nature. In TRPV subfamilies, TRPV4 is suggested to react to hypotonicity as an osmosensitive receptor and exhibits a functional role in cell-volume and fluid regulation. Therefore, if TRPV 4 is found to be present in human inner ear tissue, it is expected that it would have a role in the maintenance of osmotic pressure and fluid regulation in the inner ear, and that it may be related to the pathomachanism of Meniere's disease. However, to our best knowledge, there has been no study on TRPV 4 in the human inner ear. Thus, through immunohistochemistry, we examined whether TRPV 4 existed in the endolymphatic sacs obtained from patients with vestibular schwannoma and Meniere's disease. Further, we discuss the relationship between TRPV 4 and the pathomechanism of Meniere's disease.

Endolymphatic sacs were obtained from 4 patients with vestibular schwannoma and 3 patients with Meniere's disease. Frozen sections were immediately made after removal of each endolymphatic sac and an immunohistochemistry proceeded.

Immunoreactivity of TRPV4 was observed in the epithelium of the endolymphatic sacs of patients with vestibular schwannoma. In the endolymphatic sacs obtained from patients with Meniere's disease, degeneration was observed and epithelium seemed to have decreased. However, immunoreactivity of TRPV 4 was found to be present in the remaining epithelium.

It is suggested that TRPV4 plays a role as an osomosensitive receptor and regulates cell volume in the human endolymphatic sac epithelium. In Meniere's disease, a decrease of epithelium in the endolymphatic sac may induce disturbance of osmolality regulation and be related to forming endolymphatic hydrops.

O8

Regulation of the endocochlear potential by ATP

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Adenosine 5'-triphosphate (ATP), secreted into endolymph as sound level increases, appears to play an important role in regulating the endocochlear potential (EP) by activation of P2 receptors. Ionotropic (P2X) and metabotropic (P2Y) ATP receptors have been identified in the tissues of the compartment and ATP introduced into endolymph reduces EP in a dose-dependent fashion. The decline in EP in guinea-pig is correlated to a decline in the compartment resistance suggesting ATP activation of a cation conductance out of the endolymph (1). We propose that endogenous extracellular ATP may act to limit the driving force for sensory transduction and thus modulate hearing sensitivity and protect against loud sound by limiting hair cell activity. We conducted further studies in rat, a species in which there is more data on cochlear purinergic signalling pathways. Baseline cochlear partition resistance (CoPR) and EP were higher (5.2±0.4 kOhm and 125.3±3.9mV) than in guinea-pig (3.1±0.1 kOhm and 74.8±5.8mV). Similar to guinea-pig, injection of ATP into endolymph induced a dose-dependent decline in EP directly proportional to the CoPR decline. The recovery pattern of EP and CoPR following ATP showed some hysteresis but the direction differed between species. In the guinea-pig the EP recovers more rapidly than CoPR whereas in the rat the CoPR recovers before EP. Additional studies were undertaken to investigate the effect of exposure to a non-traumatising sound (90dBSPL BBN 3days) which has been shown to up-regulate P2X2 receptors in the cochlea. There was a substantial increase in the sensitivity to ATP introduced into the endolymph. These data confirm that ATP regulates endolymphatic electrochemical homeostasis and that the influence of ATP is enhanced following noise exposure. This adaptive response to noise exposure could contribute to sound conditioning by making the cochlea less responsive to sustained high level sound.

1. Thorne PR et al., (2004) JARO 5:58-65.

O9

Endocytosis of ion channels in the stria vascularis: Of any importance for hearing?

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It has long been accepted that marginal cells of stria vascularis are involved in the generation of the endocochlear potential and the secretion of K^+ ions (Wangemann, 1995). K^+ is the major cation in endolymph and the charge carrier for mechano-electrical transduction currents and the generation of the endocochlear potential. Accordingly several forms of hereditary deafness are due to mutations of potassium channels, including KCNQ1/KCNE1 in marginal cells of the stria vascularis (Wangemann, 2002; Jentsch et al., 2004).
Membrane recycling of apically expressed proteins is a mechanism to regulate the surface expression level of plasma membrane receptors or transport proteins. Once incorporated in endosomes, proteins can recycle back to the trans Golgi network or to the plasma membrane. If these mechanisms may also play a role for the functionally important surface expression of strial potassium channels is elusive.
Using mice with gene deletion in a most abundant lysosomal integral membrane protein type 2 (LIMP2) and mice with deletion of Megalin, the low-density lipoprotein receptor protein (LRP) we unraveled the correlation and importance of both proteins for proper strial potassium channels expression and hearing.

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O10

Expression of aquaporin-2, -3, -4, -6 and vasopressin type 2 receptor in the lateral wall of the cochlea

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Introduction: The homeostasis of water in the inner ear is essential for maintaining the function of hearing and equilibrium. Since the discovery of aquaporin (AQP) water channels, it became clear that these channels play a crucial role in inner ear fluid homeostasis. Indeed, multiple subtypes of AQPs are reported to be expressed in the lateral wall of the cochlea. However, detailed localization of AQPs and vasopressin type 2 receptor (V2-R) in the cochlea and the role of these AQPs in water homeostasis remain still obscure. In the present study, localization of AQP-2, -3, -4, -6 and V2-R in the lateral wall of the cochlea were investigated and water homeostasis of the cochlea was discussed.
Materials and Methods: Molecular biological and histochemistry studies were performed. Animals used were Wistar rats. In molecular biological study, the expression of AQP-2, -3, -4, -6 and V2-R mRNAs was investigated. In histochemistry study, localization of AQP-2, -3, -4, -6 and V2-R was observed by a Confocal laser microscopy.
Results: AQP-2, -3, -4, -6 and V2-R mRNAs were expressed in the rat cochlea. Histochemistry revealed that Proteins of AQP-2, -3, and V2-R were expressed in the basal cells on the stria vascularis. AQP-6 protein was detected in the all cells of the stria vascularis. On the other hand, AQP-4 was not expressed in the lateral wall of the stria vascularis.
Conclusion: AQP-2, -3 and V2-R is localized in the basal cells, located at the boundary between the perilymphatic system and the endolymphatic system. Since osmolality is generally thought to be higher in the endolymphatic compartment, water might enter into the extracellular space via these water channels. Water in the extracellular space is speculated to enter the marginal cells through Na-K-Cl cotransporter and to flow out through AQP-7 located in the apical membrane.

O11

In vivo visualization of endolymphatic hydrops in man

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Introduction: The recent magnetic resonance imaging (MRI) technique has made it possible to examine the cochlear compartments by using Gd-DTPA-BMA contrast agent. As the Gd- DTPA-BMA does not enter into the endolymph but loads the perilymph the technique provides possibilities to visualize the different cochlear compartments and the tightness of the endolymphatic compartment. The purpose of the study was to evaluate possible changes of the cochlea in Meniere's disease using MRI in man.
Methods: The inner ear was studied in 1.5 T MRI among four subjects with Meniere's disease. After anesthetizing the tympanic membrane 0.5 ml contrast agent was injected in the middle ear cavity. The injection took place 2 h before MRI.
Results: In all subject after 2 hours Gd-DTPA-BMA could be traced in the basal turn of the cochlea as well as in the vestibulum. The fine structure of the three partitions of the basal turn of the cochlea was visualized with MRI in three subjects as Gd- DTPA-BMA appeared mainly in scala tympani and vestibuli. In one subject the scala media was filled with Gd-DTPA-BMA. After 12 hours in one subject the Gd-DTPA-BMA had reached the apex whereas in most subjects the Gd-DTPA-BMA filled the second turn.
Conclusions: Endolymphatic space and hydrops can be visualized with high resolution MRI by using Gd- DTPA-BMA in man and it is possible to quantify the tightness of the scala media. Damage to the inner ear barrier or possible rupture of membranes can be shown with the assistance of Gd-DTPA-BMA in Meniere's disease. The use of 3T MRI will improve the image and resolution providing the possibilities for more accurate assessment of inner ear diseases.

O12

FXYD6, a novel cochlear regulator of Na,K-ATPase

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The family of mammalian FXYD proteins contains 7 members, 6 of which have been identified as regulators of Na,K-ATPase. Each FXYD protein modulates the transport properties of Na,K-ATPase e.g. its Na^+ and/or its K^+ affinity in a distinct way which is adapted to the physiological needs of the tissue in which it is expressed.
We have characterized FXYD6, the last FXYD protein of unknown function. After expression in Xenopus oocytes, FXYD6 associates with α1-β1, α2-β1, α3-β1 Na,K-ATPase isozymes. Both in non-differentiated and differentiated PC12 cells, FXYD6 can be co-immunoprecipitated with Na,K-ATPase. In Xenopus oocytes, FXYD6 slightly decreases the apparent K^+ affinity of Na,K-ATPase at slightly negative and positive membrane potentials and decreases by about two-fold the apparent Na^+ affinity. FXYD6 is mainly expressed in the brain. Interestingly, the protein is also found in the cochlea where it is expressed in various epithelial cells bording the endolymph and in the auditory neurons. Moreover, FXYD6 colocalizes with Na,K-ATPase in stria vascularis and can be co-immunoprecipitated with Na,K-ATPase. These results suggest a role of FXYD6 in endolymph homeostasis.
So in conclusion, our studies show that FXYD6 protein is a tissue-specific modulator of Na,K-ATPase. However, we cannot exclude that the FXYD6 protein may have other functions which need to be determined.

Session III: *Rescue*

O13

Animal models of salicylate and noise induced tinnitus and pharmacologic treatments

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We developed an operant technique, Schedule Induced Polydipsia Avoidance Conditioning (SIPAC), to assess salicylate, and noise induced tinnitus in rats. Acoustic overstimulation induced persistent tinnitus in some rats, transient tinnitus in others or no tinnitus. Salicylate doses between 150-300 mg/kg/d reliably induced tinnitus like behavior. Memantine, an anti-glutamatergic, and scopolamine, an anti-cholinergic, have been suggested as possible treatments for tinnitus. However, neither memantine (1.5 mg/kg) nor scopolamine (1 mg/kg), prevented salicylate induced tinnitus when given at doses that did not disrupt behavior. To speed up testing, we utilized a novel technique, Gap Pre-pulse Inhibition of Acoustic Startle (GPIAS) to assess tinnitus. The startle reflex is normally inhibited by a gap (silence) pre-pulse embedded in background noise; however, when high doses of salicylate were used to induce tinnitus, GPIAS was abolished at high frequencies (16 kHz), the presumed pitch of the tinnitus. Salicylate withdrawal eliminated the tinnitus like behavior after 1-2 days with both GPIAS and SIPAC techniques. To study the physiological mechanisms of tinnitus, 16-channel microwire electrodes were implanted in auditory cortex to monitor the local field potentials and spontaneous activity in awake rats treated with 150 mg/kg of salicylate. Local field potential amplitude increased significantly at 16-20 kHz after salicylate treatment, but recovered 1-2 days after washout. In contrast, the mean spontaneous spike rate decreased from 22 spikes/s before treatment to 14 spikes/s post-salicylate. These results suggest that salicylate induced tinnitus is associated with sound evoked hyperactivity and spontaneous hypoactivity in auditory cortex.

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O14

Acoustic stimulation via open ear hearing aid: results in tinnitus therapy

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Background
In previous papers (Tinnitus conference sept 05- ARO meeting 06) we assessed the efficacy of tinnitus treatment with sound enrichment delivered by open ear hearing instruments. We demonstrated that tinnitus patients with a hearing loss limited to f>2 kHz can perceive a significant reduction of annoyance from open ear hearing instruments monaural or binaural use after 6 month.
Aim of this research is to investigate if any audiological factor (shape of PTA, tinnitus pitch, MML, etc) has significance in therapy outcome.
Methods
35 patients who met the following requirements were recruited:
- tinnitus associated or not associated with hyperacusis;
- tinnitus associated with ski slope o mild hearing loss;
- disabling tinnitus (patients falling in category 0 according to Jastreboff cat. were excluded);
- regular follow up visits;
- open ear hearing instruments of different brands were used.
For the assessment of the efficacy of tinnitus treatment we have collected data through Jastreboff structured interview and Tinnitus Handicap Inventory Questionnaire (THI) presented upon the first visit and at the follow-up visit after a mean value of 4 months.
Results
Patients reported a very good comfort and a significant reduction of tinnitus annoyance. No statistical significant correlations were found between THI value reduction and audiological parameters.
Results will be presented in details.

Research partially granted by Tinnitus Research Initiative

O15

Prevention of noise and drug-induced hearing loss with D-methionine

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D-methionine, an amino acid and a component of fermented protein such as cheese or yogurt, markedly reduces cisplatin-, carboplatin-, aminoglycoside-, and noise-induced hearing loss in animal studies. We, and others, have obtained almost complete protection from cisplatin-induced and carboplatin-induced hearing loss without interfering with cisplatin's antitumor action. Further oral D-methionine is equally as effective as injected D-methionine for protection from cisplatin-induced hearing loss. D-met protection from aminoglycoside induced hearing loss, for both amikacin and gentamicin, is significant but partial. Further studies to optimize dosing are planned. Permanent noise-induced hearing loss in response to a 6 hour 105 dB SPL narrow band of noise can also be markedly reduced, to levels similar to control animals, whether D-methionine administration starts either before or within hours after noise exposure. We have not yet determined the maximum time delay at which D-methionine is still protective, however we have obtained significant protection/rescue even when D-methionine is first initiated up to 5 hours after noise cessation. D-methionine also protects against radiation-induced oral mucositis. The U.S. Food and Drug Administration approved our Investigational Drug Application for that application in January 2005 including the D-methionine safety data and oral drug formulation. A successful, phase 1b clinical trial for that application has been completed in India. Phase 2 clinical trials for that application in India and the US should be completed within 12 months. Phase 2 clinical trials for cisplatin and aminoglycoside otoprotection are currently in progress in India. This presentation will provide a brief overview of our translational research using D-methionine as a protective agent for both drug and noise-induced hearing loss. We are also seeking more clinical trials populations for the various applications.

O16

Drug delivery to the inner ear through a port and septum attached to the CI housing and electrode: long term in vitro safety results

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Chronic and acute drug delivery to the inner ear with a cochlear implant can be achieved through a titanium port with septum. The port is fused with the implant housing (PULSAR CI100) and the chamber is connected to a channel within the electrode array. The essential safety requirement is for the septum to be leak proof for the expected life of the implant (>20 years). Nine ports with septum designed for CI drug delivery have been tested in accelerated conditions for leak profess. The septum's were perforated 25 times with a 30 gauge needle. The needle was left in place for 30 days at 37 C in saline solution. The needle was then removed. Pressure of up to 2.5 bars was applied to the back end of the port to examine if air bubbles were expelled through the saline. Subsequently the ports were placed in saline at 100 C and periodically tested for leakage. Design of the cochlear implant with drug delivery and results of the aging test will be presented up to conference time. Additional safety concerns that have been tested will be shown (biofilm formation, histology, impact test). The concept of safe fluid drug delivery to the inner ear will be addressed.

O17

Local administration of triamcinolone or dexamethasone preserve hearing after cochlear implantation in guinea pigs: CAP-measurements

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Combined electric-acoustic stimulation (EAS) of patients with residual hearing has already leaded to an improvement of speech understanding. Therefore it is important to preserve hearing during implantation and afterwards as much as possible. Since corticosteroids have been successfully administered in patients with various hearing impairments it is safe to assume that they can also attain a benefit for hearing after cochlea implantation.
In the present study two different corticosteroids were administered directly to the cochlea of guinea pigs in order to analyse possible differential effects on hearing preservation after cochlea implantation.
Three groups of guinea pigs were implanted with guinea pig electrodes (supplied by MED EL) through a cochleostomy in the basal turn of one cochlea. Either triamcinolone, dexamethasone or artificial perilymph (AP) was infused with a micro-syringe directly before implantation. The other ears were infused equally (omitting implantation) and served as an additional control. Click-evoked compound action potentials (CAPs) and frequency-specific CAP-audiograms were recorded using RW electrodes. Hearing loss (HL) was measured before and after drug/AP application and during the following 3 months.
HL was most pronounced in implanted ears treated with AP. CAP-audiograms revealed lower threshold shifts at all frequency ranges for steroid-treated animals. The time course for the protective effect was different for the two drugs. Efficacy of dexamethasone best in the course of three weeks after implantation declining afterwards. In contrast triamcinolone worsened hearing during the first 3 days post-implantation but revealed an improvement from day 7 and afterwards.
The results indicate that the two steroids reduce hearing loss caused by cochlear implantation even after a single application. Dexamethasone probably requires a repeated or sustained application to extend protection over a longer space of time.

Supported by DFG & MED EL.

O18

TNF alpha is ototoxic to the hair cells of organ of Corti explants and dexamethasone protects these sensory receptor cells from damage by TNF alpha

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TNF alpha plays a major role in endoplasmic reticulum stress-induced cell death (Yang et al., 2006). Dexamethasone inhibits TNF alpha-induced cell death (Udo et al., 2001). TNF alpha is expressed in the cochlea following vibration induced hearing loss (Zou et al., 2005). There is no direct proof that TNF alpha is ototoxic to hair cells (HCs).
To explore the effect of TNF alpha on HCs and the protective effect of dexamethasone (DXM) we used P-4 rat organ of Corti explants and a series of TNF alpha conc. (i.e. 25 to 500 ng/mL) and a DXM conc. of 750 micro-g/mL in the medium. After 4 DIV, explants were fixed, stained with FITC-phalloidin and examined with fluorescence microscopy. Cuticular plates with stereocilliary bundles were counted as intact HCs and expressed as HC density. Hair cell counts were expressed as mean values +/- SD. Statistical analysis was performed with a Students t test and ANOVA; significance was set at $p < 0.05$.
The loss of auditory HCs in the TNF alpha exposed explants occurred in a dose dependent manner with the loss of HCs expressed in a characteristic base to apex pattern. Loss of HCs in the explants began at a TNF alpha conc. of 50 ng/mL. The viability of both inner (IHCs) and outer (OHCs) hair cells was affected by ototoxic levels of TNF alpha. The presence of DXM in the medium containing an ototoxic dose of TNF alpha (i.e. 125 ng/mL) protected both IHCs and OHCs.
TNF alpha is ototoxic to HCs in organ of Corti explants and dexamethasone protects the explant's HCs from TNF alpha's ototoxicity. Based on these in vitro results and those of the vibration-induced hair cell death study (Zhou et al., 2005), TNF alpha may be involved in the loss of HCs that are subjected to a physical trauma such as can occur during skull base surgery and cochlear implantation. Locally delivered dexamethasone may be an effective therapy for the conservation of hearing.

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Session IV: *Ototoxicity and Physiopathology*

O19

Phosphoinositide signaling and cytoskeletal arrangements in the organ of Corti

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Aminoglycosides strongly bind to phosphoinositides and affect their membrane distribution and metabolism. Phosphoinositides, in turn, regulate the cellular cytoarchitecture via direct interactions with cytoskeletal components or via signaling pathways mediating actin organization. Here, we investigate phosphoinositides, small GTPases and actin in the mouse inner ear in vivo with chronic systemic administration of kanamycin as a probe, the formation of F-actin was impeded, the arrangement of beta-actin was changed in the stereocilia of outer hair cells, and the intermittent adherens junction/tight junction complexes were distorted between outer hair cells and supporting cells.
The drug treatment concomitantly activated Rac1, promoted the formation of the complex of Rac1 and p67phox, decreased the activity of RhoA, and reduced the formation of the RhoA/p140mDia complex. Immunoreactivity to phosphatidylinositol-3,4,5-trisphosphate (PIP3) decreased in the organ of Corti, especially in outer hair cells, while phosphatidylinositol-4,5-bisphosphate (PIP2) increased. In agreement with reduced PIP3 signaling, phosphorylated Akt1/2 decreased in both the cytoplasm and nuclei of outer hair cells after kanamycin treatment. PIP2 was initially present at the apical poles of outer hair cells but appeared in their nuclei after drug treatment. Nuclear PIP2 formed a complex with histone H3 and attenuated its acetylation.
These findings suggest that kanamycin activates small GTPases via the disturbance of membrane microdomains and controls the arrangement of the actin cytoskeleton. In addition, activated GTP-Rac may disturb the balance between PIP2 and PIP3, modify gene transcription via histone acetylation and diminish the PIP3/Akt survival pathway. These actions may contribute to the death of outer hair cells.

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O20

Vestibular toxicity of nitriles in the mouse and the guinea pig

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Several nitriles have been demonstrated to cause hair cell loss in the rat, but the susceptibility of other species to this toxic effect has not been investigated. Male adult Swiss mice were orally dosed with control vehicle, cis-crotononitrile (2.75 mmol/kg) or 3,3'-iminodipropionitrile (IDPN, at 8, 16, and 24 mmol/kg) (n=7/group). The mice were then assessed for behavioral effects up to day 21 post-dosing, using a test battery sensitive to vestibular dysfunction. Both IDPN and cis-crotononitrile were observed to cause a persistent loss of vestibular function; the effect of IDPN was dose-dependent. Hair cell loss in the vestibular sensory epithelia was assessed at 5-12 weeks by scanning electron microscopy (SEM). Treated mice showed missing hair bundles, up to a complete loss in the most affected animals. Hair cell loss following IDPN was dose-dependent, with a good correlation with the behavioral data. Consistency between behavioral evidence of vestibular dysfunction and bundle loss was also recorded on an animal to animal basis. Further experiments were done with male albino guinea-pigs (350-400 g). After receiving IDPN (0, 1.6, 2.4 or 3.2 mmol/kg, i.p., n=2/group), the guinea pigs developed behavioral abnormalities indicative of vestibular dysfunction, with more overt effects observed in the animals treated with larger doses. SEM observations at 4 to 6 weeks after treatment, demonstrated a dose-dependent loss of hair bundles. In conclusion, the present study demonstrates that the nitriles known to cause hair cell degeneration in the vestibular system of the rat are also effective in the mouse and the guinea pig. This opens the possibility of using genetically modified and mutant strains of mice to investigate the basis of this toxicity, and of using these nitriles for vestibular research conducted in guinea pigs.

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O21

Thrombomodulin expression in the stria vascularis

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Inner ear diseases, sudden deafness, Meniere's disease and other conditions have been suggested as the cause of insufficiency of cochlear blood flow. However little is known about the regulation of cochlear blood flow. Suppression of capillary thrombosis is thought to be important in the stria vascularis, since its blood flow is believed to be slower than in the spiral ligament. In this study, we examined the expression of thrombomodulin, which suppresses thrombosis, and its altered expression after endotoxin or sound treatment.
EXPERIMENTAL DESIGN: We have examined the expression of thrombomodulin in surface preparations and sections of the stria vascularis of rats and mice using immunohistochemical methods and RT-PCR. 1) To investigate the effect of endotoxin, normal saline or 5mg/ml lipopolysaccharide was delivered via the round window membrane. 2) To investigate the effect of sound stimulation, animals were exposed for 24 hours to a 120 dB SPL band noise. They were then sacrificed and examined for thrombomodulin expression.
RESULTS: Thrombomodulin was found on the capillary surface of stria vascuralis in the sections of rat and mouse cochlea. In the surface preparations, thrombomodulin was also detected on the capillary surface of stria vascuralis, but in small amounts. In contrast, higher expression of Thrombomodulin was frequently recognized in the specimens from the animals treated with LPS or sound than in those treated with normal saline.
CONCLUSIONS: Thrombomodulin exists in the capillary of stria vascularis, and may be related to the suppression of capillary thrombosis in the cochlea. Expression of thrombomodulin is increased during chemical and physical stimulations, thereby likely producing anticoagulation effects under these conditions.

O22

The effects of several anaesthetics on auditory evoked compound action potentials and brainstem responses in the guinea pig

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Safety regulations suppress the use of halothane as an anaesthetic. The effects of alternatives on auditory evoked compound action potentials (CAP) and brainstem responses (ABR) in the guinea pig are poorly known. We started a study on these effects with three different anaesthetics at several doses.
We used guinea pigs with permanently implanted electrodes, allowing recording in the awake condition and under anaesthesia in the same individuals. A period of a week was given between successive experiments in individual animals. Core temperature of the animals was kept constant at 38 degrees Celsius.
We used ketamine, in combination with xylazine, intramuscularly at doses of 20, 40 and 80 mg/kg, isoflurane, at 2 to 3 % in a gas mixture containing N2O and O2 (2:1) and pentobarbital, intraperitoneally at 20, 40 and 80 mg/kg.
Ketamine produced a frequency dependent increase in CAP thresholds, up to 15 dB at 16 kHz. CAP latencies were increased by around 0.2 ms. Dose was not a significant factor. ABR latencies were also increased. The increase was 0.2 ms for peak I and around 0.3 ms for peak IV.
Isoflurane produced comparable results, but the frequency dependence of CAP threshold shifts was less clear. Also, latency shifts for both CAPs and ABR peaks were larger than for ketamine, e.g., ABR peak IV latency increased by 0.6 ms at 3% isoflurane.
Pentobarbital produced highly unreliable anaesthesia at 20 and 40 mg/kg and killed the animals at 80 mg/kg. Despite these difficulties, significant latency shifts for ABR peaks were found.
In conclusion, ketamine, isoflurane and pentobarbital all have an effect. Mechanisms may involve middle ear effects, effects in the cochlear periphery and more central effects. The frequency dependence of some of the effects suggests that the neuropharmacological pathways are different for different frequencies. Isoflurane and ketamine are reliable anaesthetics at the doses used here, but pentobarbital is unsuitable in guinea pigs.

O23

Presbycusis – a complex disorder with genetic and environmental background

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Purpose: Previous epidemiological studies suggest that in presbycusis hereditary component may exists behind the hearing loss (HL). The role of biological and environmental components is not well understood. We aimed to study the role of environmental and biological variables in the etiology of presbycusis.
Methods: We evaluated 1548 cases entered for hearing aid fitting in tertiary health service. The patients filled in a questionnaire concerning their general health, free time and occupational noise exposure, diseases, medication and the possible susceptibility factors for HL. Patients with ear diseases (n=166) were excluded from the analysis.
Results: The mean age of the patients was 74,6 years, 55,5 % were females and 44.5 % males. The average HL in speech area was 48,8 dB in the right and 49,7 dB in the left ear. Totally 42,5 % had noise exposure exceeding Leq 80 dB(A) and 14,5 % had noise exposure exceeding Leq 90dB. In linear regression analysis environmental noise explained 5% of the variability and the only significant environmental variable was free time shooting not occupational noise. Concerning biological factors age, cholesterol treatment and blood pressure treatment were significant determinants. Neither smoking nor use of analgesics was significant in the genesis of HL. The hereditary factors were statistically significant determinant for the extent of HL. From diseases linked variables migraine, Raynaud's phenomenon and transient ischemic attacks were significant determinants for HL.
Conclusions: The HL for presbycusis seem not to be and additive component for noise-induced hearing loss and nosocusis, although some interaction occurs. At old age the role of environmental factors and general health are less important factors. Neither smoking nor use of analgesic is significant predictors for HL. Genetic background seems to be the main determinant for the HL at old age.

Session V: *Mechanisms of Development and Repair*

O24

Brief noise exposure in juvenile rats causes a deterioration in the frequency selectivity of inferior colliculus neurons in adulthood

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Previous studies in rats and mice have demonstrated that in the central auditory nuclei, low-frequency response domains develop earlier than those sensitive to high frequencies. Thus, brief exposure to intense sound during a sensitive period of postnatal development can alter neuronal excitability in the auditory system of adult animals and results in poorer frequency selectivity of neurons recorded in central auditory nuclei. In this study, juvenile rats on postnatal day 14 were exposed to a broad-band noise of 125 dB SPL intensity for 8 minutes. Later, at the age of 3-6 months, the frequency tuning curves of individual IC units were measured in these rats (anaesthetized by a ketamine-xylazine mixture) and their widths 10, 20 and 30 dB above threshold (Q10, Q20 and Q30 parameters) were determined. The results were compared with those obtained in control animals of the same age. The brief exposure of juvenile rats to intense noise produced only temporary hearing threshold shifts, occurring mainly at high frequencies. The thresholds of individual IC neurons in adult animals were similar to those obtained in the control unexposed group. The IC tuning curves measured in acoustically primed rats and in age-matched controls demonstrated a similar sharpness in neurons with a characteristic frequency lower than 16 kHz. However, IC neurons with a characteristic frequency higher than 16 kHz had significantly lower Q parameters than did those in age-matched controls, indicating poorer frequency selectivity. In addition, in many high-frequency neurons in exposed animals, a ‘notch’ in the IC frequency tuning curves between 10-25 kHz was observed. The results demonstrate that short, intense noise exposure in juvenile rats can have an impact on the frequency selectivity of adult animals, resulting in a significant worsening of the frequency-tuning properties of individual high-frequency IC neurons.

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O25

Expression of genes involved in the apoptosis in the organ of Corti, the modiolus and the stria vascularis of newborn rats

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This report presents data on the expression of genes involved in the apoptosis in the inner ear of newborn rats, based on microarray and quantitative RT-PCR data. Organ of Corti (OC), modiolus (MOD) and stria vascularis (SV) were prepared from neonatal rat (3-5 days old) and analyzed immediately after preparation and 24 h after cultivation. Pooled samples of the following cochlea preparations were studied: (1) native preparations as controls, (2) organotypic cultures under normoxic conditions, (3) cultures which were exposed to 5 h hypoxia during cultivation.

The basal expression in control samples of all regions comprised a high level of expression of superoxide dismutase 1 (SOD-1), Bax and Calpain-1. Cultivation induced clear region-specific changes in gene expression. In the OC, cultivation induced down-regulation of the pro-apoptotic genes caspase-2, -3, -6 and calpain-1. In the MOD, besides the decrease of caspase-2 induction, cultivation induced up-regulation of the anti-apoptotic SOD-2 and SOD-3. In the SV, cultivation induced changes observed in the OC (down-regulation of caspase-2, -3, -6 and calpain-1) and changes observed in MOD (increase of SOD-2).These changes may reflect effective adaptational activities ensuring the survival of cells under in vitro conditions.

In hypoxia, only two genes responded statistically different as compared to cultivation: (1) SOD-2 mRNA levels further increased in the MOD and (2) caspase-6 increased in the OC, possibly indicating a regulatory role in hair cell death.

It is concluded from these findings that simultaneous changes of several species of genes are required in order to bring about a significant protection of the inner ear cells. Thus, new therapeutic strategies targeting several protective factors may prove more efficacious than selectively inhibiting a single factor.

O26

Sox 10 is not necessary for auditory neurons survival

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The inner ear is a complex 3D structure that derives from an ectodermel thickening, the otic placode. Classical studies established that the cochleo-vestibular ganglion (CVG) also derives from this otic placode. The glial cells of this CVG come from another embryonic structure, the neural crest stem cells. In this work, based on a mouse that carries a mutation of Sox10 (Sox10LacZ mouse), we demonstrate that the auditory neurons of the spiral ganglion (who derive from the CVG) are able to survive without Sox10 and without glial cells. Sox10 is a transcription factor that plays an important role in the neural crest development and is a key regulator in differentiation of peripheral glial cells. Deprivation of Sox10 in the peripheral nervous system ganglia results in neurons death. In the Sox10lacZ mouse, neuronal cells form in dorsal root ganglion and in the enteric nervous system, but glial cells (Schwann cells or satellite cells) are not generated. At later developmental stages, this lack of peripheral glial cells results in a severe degeneration of neurons. In the spiral ganglion (of placodal origin) of these mice, we showed that there is a lack of glial cells. We also demonstrated that this spiral ganglion contains neurons and that these neurons have no histological signs of degeneration or necrosis. These results support the idea that the survival of spiral ganglion neurons is Sox10 and glial cells independant.

O27

P2X2/3 receptor inhibition of BDNF-mediated spiral ganglion neurite outgrowth

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Type I and type II spiral ganglion neurons (SGNI and SGNII) project to both inner and outer hair cells in the rat in the first post-natal week. This entails substantial synaptic reorganization whereby OHC SGNI innervation is lost, SGNII is established, and exclusive innervation of the IHC by multiple type SGNI neurites undergoes pruning of synaptic branches to achieve an adult-like morphology. The transient expression of P2X3 receptors (ATP-gated ion channels) coincides with this process. However, the SGN ATP-gated ion channel phenotype is incompatible with the P2X3 expression. Real-time RT-PCR was used to analyse the relative levels of transcript expression for seven different P2X receptor subunits in individual post-natal rat SGN (P2 – P4). The data revealed a preponderance of P2X2 and P2X3 transcripts in a 1:2 ratio, supporting the expression of P2X2/3 heteromeric ATP-gated ion channels. In addition, expression of recombinant P2X receptors in Xenopus oocytes highlighted the contribution of a spliced variation of the P2X2 receptor to the pharmacology of the ATP-gated ion channel established in SGN. To ascertain the functional significance of these ATP-gated ion channels, spiral ganglion explants were cultured for three days, with or without BDNF to promote neurite outgrowth via the TrkB signalling pathway, as a model for cochlear neural development. ATPgS or a,b,meATP - hydrolysis resistant ATP analogs which activate P2X2/3 receptors - were applied (100 µM). These ATP analogs had no independent effect on the number or length of the SGN neurites. However, they did significantly inhibit BDNF-derived outgrowth. These data suggest that purinergic signalling in SGN during a critical period just prior to the onset of hearing may contribute to the pruning of neurite connections with hair cells - a key element in the maturation of the cochlear afferent innervation.

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O28

Peripheral nerve regrowth in vivo through BDNF/FGF treatment in deafened guinea pigs

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Introduction: Loss of the receptor cells in the mammalian cochlea results in a retrograde degeneration of peripheral processes and in turn spiral ganglion neurons.
This may impinge upon the efficacy of the cochlear implant in human and reduce specificity of electrical stimulation of these prostheses.
Hair cells seem to provide SGCs permanently with nutritive growth factors, so that hair cell loss consequences in degeneration of SGCs. The effects of neurotrophic factors (NTF) to prevent degeneration of neural elements have been shown in animal experiments.
Methods and Discussion: Studies were performed in guinea pigs deafened with kanamycin (450mg/kg) SQ 2 hours prior to ethacrynic acid (60mg/kg). Baseline aABR confirmed deafness. BDNF and aFGF were applied locally via mirocannula-osmotic pump to the scala tympani. Control groups included normal hearing animals and animals that survived 3 days to 7 weeks post deafening.
In this study we examined morphological and quantitative changes of primary neurons in the Rosenthal´s canal and of their peripheral processes following deafness. The mean density and diameter of primary neurons was assessed for each turn. Influence of NTF treatment post deafening after short (3days) and long (3 weeks) delay on peripheral processes and SGN are shown using immunohistochemistry for efferent and afferent fibers in the cochlea at both light- and electron microscopic level. Afferent and efferent nerve endings were quantified using confocal microscopy and BDNF/aFGF treated and not treated side within the same animal compared. Confocal data of double-stained whole mount preparations and light microscopic sections demonstrate the afferent-efferent degeneration pattern and regrowth of afferent fibers in NTF treated animals.

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O29

Evidence for the involvement of JNK signaling in epithelial repair, proliferation and stereocilia development during hair cell regeneration

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The role of JNK signaling in hair cell death has become well known, but JNK is also involved in numerous other processes, such as cell proliferation, embryonic dorsal closure and non-canonical Wnt signaling. We investigated the possible role of JNK activation during sensory regeneration in the avian inner ear. One set of experiments was carried-out on cultures of isolated sensory epithelia from the chick utricle. A laser microbeam was used to create 'wounds' in confluent epithelia and specimens were examined after 0.5-24 hours recovery. In normal (control) cultures, we usually observed complete wound closure after 24 hours. Laser wounding also resulted in a rapid activation of JNK, as revealed by numerous cells expressing phosphorylated c-Jun near the lesions. Inhibition of JNK signaling (with the inhibitor SP600125) prevented epithelial repair in about 90% of the specimens. Additional experiments examined the effects of JNK inhibition on proliferation in primary cultures of cochlear and utricular supporting cells. Treatment with two different JNK inhibitors – SP600125 and CEP-11004 – resulted in reduced proliferation of both cell types, while treatment with SU-5402 (an inhibitor of FGF-R) had no effect on proliferation. Finally, a third set of experiments examined the role of JNK activation during hair cell differentiation. Chick utricles were cultured for 24 hours in 1 mM streptomycin, which killed nearly all existing hair cells. Specimens were then cultured for an additional 10 days, in order to permit regeneration. Some specimens received the JNK inhibitor SP600125 for the final six days in culture (the period corresponding to hair cell differentiation). Inhibition of JNK had no effect on hair cell differentiation (as assessed by calretinin immunoreactivity), but did inhibit the development of stereocilia bundles. Together, the results suggest that JNK signaling is involved in several independent processes during hair cell regeneration.

Session VI: *Deafness Genes*

O30

Progression of the inner ear pathology of DDR1-null mice

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Discoidin Domain Receptors 1 and 2 (DDR1 and DDR2) are tyrosine kinase receptors activated by native collagen. There physiological functions are in cell growth, adhesion, migration and matrix homeostasis. Aberrant expression and signaling of DDR1 and DDR2 have been implicated in several human diseases linked to accelerated matrix turnover including tumor invasion, atherosclerosis and tissue fibrosis. Amongst other phenotypes, DDR1 knockout mice present with proteinurea due to aberrant morphology of the renal glomerular basement membrane and as we showed preliminary also a phenotype in the inner ear, however, the role of DDR1 in the development and function of the inner ear has not been elucidated. Here we describe, using conventional and pre-embedding immunohistochemistry, an expression of DDR1 protein in several locations in the cochlea mostly associated with basement membrane collagens and with cells containing contractile elements such as outer hair cells and ultrastructurally a phenotype of the DDR1-null mice comparing one, two and four month old animals. Decrease of the auditory function in DDR1-null mice is associated by substantial structural alterations in the inner ear tissue. We observed a progressive alteration of the several cells types of the cells lining the basilar membrane including the organ of Corti. Deiter's cells showed reduced attachment to the basilar membrane, while outer hair cells had alteration of the lateral wall. Confocal microscopy was used to identify a candidate for cytoskeleton interaction, however, a motor molecule- prestin still identified in the detached lateral wall of the outer hair cells. These alterations may directly interfere with motile properties of the organ of Corti and be responsible for the hearing loss observed in DDR1-null mice.

O31

Hearing loss in mice with partially-deleted vitamin D receptor gene

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Hearing loss associated with vitamin D insufficiency and hypervitaminosis is well-known clinically. Here we describe that lack of vitamin D action in vitamin D receptor (VDR)-deficient mice causes hearing loss. Forty three adult male and female mice [24 VDR-/- and 19 wild type(WT)] were used in the studies. To eliminate hypocalcaemia and rickets in the VDR-/- mice, all mutant animals were fed a special rescue diet containing 2% Calcium, 1.25% Phosphorus and 20% lactose. Click ABR was measured at 3-9.5 months age. JB4 embedding and 2 µm thin sectioning was used to evaluate the cochlear morphology. Immunofluorescent confocal microscopy was used to analyse the protein expressions of synaptophysin, connexin 26, KCNJ10, TRPV4 and TRPV6. Cochlear connexin 26, KCNJ10, TRPV4 expression were not affected in the VDR-/- mice. TRPV6 was not consistently expressed even in the WT mice cochlea. The hearing loss is caused by abnormal differentiation of the outer hair cells (double layered outer hair cell-like cells without fully developed cellular architecture) in the cochlea, which in turn causes an interrupted peripheral nervous signalling, although the central auditory system may also be impaired (possibly thalamic calcification and auditory system demyelination). It will be important to include vitamin D status in the routine clinical examination of pregnant woman to prevent possible congenital sensorineural hearing loss.

O32

Novel pendrin and myosin VI mutations in humans and mice: functional implications

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New alleles of known deafness genes are providing us with novel phenotypes that may help elucidate the function of the proteins these genes encode. The solute carrier family 26, member 4 (SLC26A4) gene encodes the pendrin protein, mutations in which lead to Pendred syndrome (PDS), as well as nonsyndromic forms of deafness. Myosin VI (MYO6) mutations in humans lead to nonsyndromic hearing loss and is associated with cardiomyopathy as well.
N-Ethyl-N-Nitrosourea (ENU) mutagenesis screens provide new mouse models for human hearing impairment, a requisite for studying the complexity of inner ear development and mechanisms of human mutations. Two new ENU mouse mutants, loop and Tailchaser, are profoundly deaf according to the auditory brainsteam response (ABR) test. The mice exhibit a wide range of defective vestibular defective behavior. In loop, a recessive mutation, a closer look at the vestibular sensory organs revealed a giant stone structure overlying the vestibular macula instead of the tiny scattered otoconia. In the organ of Corti, abnormalities are seen in the marginal pillar cells. Chromosomal mapping localized loop to chromosome 12. A novel missense mutation was subsequently identified in the Slc26a4 gene. In Tailchaser, a dominant mutation, scanning electron microscopy revealed that the mice show distinct changes in their hair bundle morphogenesis that includes variable positioning of the kinocilium and stereocilia fusion. The Tlc locus was mapped to mouse chromosome 9. A novel missense mutation was subsequently identified in the myosin VI gene. Functional assays revealed the mechanisms of each mutation. Comparison with novel mutations identified in the human population will help shed light on elucidating genotype-phenotype correlations.

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O33

Hearing impairment in an animal model of oculocutaneous albinism type I

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Albinism has been associated to hearing impairment in a variety of species but the molecular bases for this association are not entirely understood.
To explore if cochlear hypo-pigmentation might cause or contribute to hearing alterations we have studied the auditory function in a genetically well-defined mouse model of oculocutaneous albinism type I (OCA I) (1). OCAI, a common form of albinism, is caused by mutations in the tyrosinase gene. To further study the role of tyrosinase in ear development and hearing, we have evaluated the auditory function of transgenic mice expressing a functional genomic-type tyrosinase construct, and hence pigmented. Albino and pigmented mice only differ in the presence of the tyrosinase transgene that is responsible for rescuing the albino phenotype, and thus tyrosinase-transgenic mice are undistinguishable from wild-type pigmented mice.
Both pigmented and albino animals have been analysed by testing the auditory brainstem response (ABR) at different ages under different conditions. ABR studies were performed as reported (2; http://www.iib.uam.es/servicios/nine/intro.es.html).
Our results suggest that albino mice present an advanced age-related hearing loss and increased sensitivity to noise damage as compared with the pigmented animals.

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O34

Contribution of GJB6 to DFNB1 related hearing loss in Portuguese families

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Deafness-causing point mutations in the Cx30 gene, GJB6, are very rare, unlike the neighbor GJB2 gene, encoding Cx26. However, large deletions in GJB6, associated to hearing loss, have been found in several populations and may be an important cause of non-syndromic recessive deafness.Del(GJB6-D13S1830) deletion, encompassing 309kb, was first described in 2001 and includes the 5' region of GJB6 and most of gene Cryl I, with no known relation with hearing. The deletion was mainly found in patients heterozygotes for recessive mutations in GJB2, thus contributing to elucidate the cause of deafness in these individuals. A multi-center study involving patients from nine countries, revealed that del(GJB6-D13S1830) deletion is present in most of the screened populations, but with different prevalence, pointing to a common founder in Western Europe countries. Since then, many research groups all over the world have been screening their patients, carrying one or null GJB2 mutant allele, for its presence.
A second deletion was recently found. The del(GJB6-D13S1854) encompasses 232kb, including the 5' region of GJB6 and half of Cryl I gene. Again, a multi-center study found variable frequencies of this second deletion around Europe, and concluded that a probable common founder for the mutation occurred in Spain, Italy, and the UK. In those countries where del(GJB6-D13S1854) was detected, it helped to solve un-elucidated cases of carriers of GJB2 mutations or of del(GJB6-D13S1830).
In this study, we summarize the results obtained for over 100 unrelated cases from the Portuguese deaf population regarding the detection of del(GJB6-D13S1830) and del(GJB6-D13S1854) in individuals with a single or no mutation in GJB2. No del(GJB6-D13S1830) carrier was detected. On the contrary, four unrelated deaf individuals were found to be carriers of del(GJB6-D13S1854), thus leading us to the assumption that this deletion may be an important cause of deafness in the Portuguese population.

O35

Catalase polymorphisms associated with noise-induced hearing loss in two independent noise-exposed populations

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Noise-Induced Hearing Loss (NIHL) is one of the most important occupational diseases. It is assumed that 400 to 500 million people in the USA and Europe are daily being exposed to harmful noise levels. NIHL is a complex disease resulting from an interaction between genetic and environmental factors. Although the environmental risk factors have been studied quite extensively, little is known about the genetic factors. Based on multiple studies, it was proposed that oxidative stress plays an important role in the development of NIHL. Here, we investigated whether variations (Single Nucleotide Polymorphisms; SNPs) in the catalase gene, one of the genes involved in oxidative stress, influence noise susceptibility. Audiometric data from 1261 Swedish and 2500 Polish noise-exposed labourers were examined. DNA was extracted from the 10 % most susceptible and the 10 % most resistant individuals. Twelve SNPs were selected and genotyped. Subsequently, the interaction between noise exposure and genotypes, and their effect on NIHL were statistically analysed using the logistic regression test. Interactions between genotypes of three SNPs for the Swedish population and of two SNP for the Polish population and noise exposure levels were significantly associated with the susceptibility to noise. No main effect was found for these SNPs. One other SNP had a significant main effect in the Polish population. The interaction of haplotypes and noise exposure levels and their effect on NIHL was also analysed, resulting in several significant associations. In conclusion, this study identified significant catalase-noise exposure interactions, influencing the susceptibility to noise and the development of NIHL, indicating that catalase is the first NIHL susceptibility gene.

Session VII: *Micromechanics*

O36

The outer hair cell active force production in the cochlear environment

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Cochlear outer hair cells amplify and sharpen acoustic signals, and they are crucial to active hearing in mammals. It has been demonstrated that isolated outer hair cells produce electromotility-based constant active forces up to several tens on kilohertz. It is of key importance to understand how the outer hair cell generates physiologically significant active forces under high-frequency conditions in the cochlear environment. In the cochlea, the cell active behavior is modulated by mechanical (surrounding viscous fluid, deformable basilar and tectorial membranes) and electrical (cell's membrane and intracochlear potentials) factors. We present computational modeling of the outer hair cell active force production that takes into account the electrical, electromechanical (prestin-related piezoelectricity, mechanosensitive channels), and mechanical (stiffness and viscosity) properties of the cell's composite membrane as well as the cell interaction with the cochlear fluid and solid components. Our analysis also includes the effect of the intracochlear electric potentials measured under high-frequency conditions in the vicinity of outer hair cells. The parameters of our integrative model are based on our experimental data as well as the measurements available in the literature. We have shown that an individual outer hair cell can produce a force of several tens of pN for frequencies up to several tens of kHz. The obtained results are important for a better understanding of the role of outer hair cells in the mechanism of cochlear amplifier.

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O37

Membrane tension effects on chloride binding to prestin

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The motor protein, prestin, is responsible for our exquisite sense of hearing by providing the basis for cochlear amplification. Chloride is an important modulator of prestin activity since it binds to this molecule and alters characteristics of the motor's displacement charge, or nonlinear capacitance (NLC; Oliver et al., 2001; Rybalchenko and Santos-Sacchi, 2003; Song et al 2005). Indeed, chloride has been recently shown to directly alter cochlear amplification in vivo (Santos-Sacchi, 2006). In addition to prestin's anion sensitivity, motor charge also responds to changes in membrane tension. Here we report on preliminary studies to determine whether membrane tension affects prestin's binding affinity for anions. Isolated OHCs were whole cell voltage clamp and NLC was measured under conditions of different intracellular turgor pressure while varying salicylate concentrations, an anion that competes for prestin's chloride binding site. Positive membrane tension induced by turgor pressure was found to shift the dose response curve of salicylate's reduction in NLC, indicating an increase in the IC_{50} for salicylate. This can be interpreted as a reduction in the affinity of salicylate for prestin, and may account for tension effects on OHC performance. Further studies on tension's effects on chloride bind are underway. We hypothesize that the conformational changes in prestin which occur during imposed membrane tension alters the binding site for anions.

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O38

Non-mammalian orthologs of prestin are electrogenic divalent/chloride exchangers

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Individual members of the mammalian SLC26 anion transporter family serve two fundamentally distinct cellular functions. Whereas most members transport different anion substrates across a variety of transport epithelia, prestin (SLC26A5) is special, functioning as a membrane-localized (integral) voltage-controlled motor protein that generates electrically induced motions (electromotility) in auditory sensory hair cells of the mammalian inner ear. To address a potential mechanistic relation between anion transport and electromotility, we have cloned prestin orthologs from chicken (cPres) and zebrafish (zPres), non-mammalian vertebrates that presumably lack electromotility in their auditory system. We examined possible anion transport by electrophysiological measurements from CHO-cells and Xenopus laevis oocytes heterologously expressing cPres or zPres. In both systems proteins were GFP-tagged to permit checking of delivery to the plasma membrane. Here we show that the non-mammalian prestin orthologs are electrogenic antiporters, exchanging sulfate or oxalate for chloride in a strictly coupled manner with a 1:1 stoichiometry. This transport is blocked by salicylate, a known inhibitor of electromotility. In contrast, electrogenic divalent transport was not detectable in mammalian prestin. The high degree of sequence conservation between mammalian and non-mammalian prestin together with a common pharmacology of electromotility and divalent antiport strongly suggest that the molecular mechanism behind electromotility is closely related to an anion transport cycle.

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O39

A low-coherence interferometry system and its use for vibration measurement and imaging of the guinea pig organ of Corti in vivo

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An optical coherence tomography (OCT) system was built to acquire in vivo, both images and vibration measurements of the organ of Corti of the guinea pig. A standard time-domain OCT system structure was adopted, using a scanning phase delay line in the reference arm to achieve longitudinal z-axis scanning and a galvo-mirror in the sample arm for lateral x-axis scanning. The organ of Corti was viewed through a ~300µm diameter hole in the bony wall of the cochlea at the scala tympani of the first cochlear turn. In imaging mode, the image was acquired as reflectance R(x,z). In vibration mode, the basilar membrane (BM) or reticular lamina (RL) were selected by the investigator interactively from the R(x,z) image. Under software control, the system would move the scanning mirrors to bring the sensing volume of the measurement to the desired membrane location. In vivo images of the organ of Corti clearly indicate strong reflectance signals from the BM, RL, tectorial membrane (TM), and Reissner's membrane (RM). The tunnel of Corti and the inner sulcus are also visible in the images. The vibration responses of the BM, RL, TM and RM from an insensitive guinea pig cochlea were measured by directing the scanning mirror to the corresponding locations. The ratio of vibration displacement spectra of these measured membrane to that of the stapes was calculated. The noise floor of current vibration measurement is less than 0.2 nm, measured within the frequency range of 5 kHz to 22 kHz. The results shown demonstrate the unique optical sectioning capability of this system, which provides a powerful tool for studying the cochlear micromechanics.

O40

Group delay contour plots derived from human DPOAE level/phase maps

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Distortion-product otoacoustic emission (DPOAE) level/phase maps similar to those described by Knight and Kemp (2000) were collected with the DPOAE frequency range extended to lower frequencies in 20 normal-hearing subjects (10 females, 10 males) and 10 males with noise-induced hearing loss (NIHL). To construct the DPOAE ratio vs level and phase plots, DPOAEs were measured in DPOAE frequency steps of approximately 44 Hz from 0.5-6.0 kHz in response to primary-tone sweeps at three levels (80,80; 75,75; 65,55 dB SPL), using constant f2/f1 ratios incremented in 0.025 steps from 1.025-1.5. DPOAE level was directly plotted while the phase was corrected for primary-tone phase variation and unwrapped before plotting. The 2f1-f2 DPOAEs showed “wave-fixed” phase patterns at standard f2/f1 ratios of 1.21 and “placed-fixed” phase behavior for closely spaced f2/f1 ratios. The 2f2-f1 DPOAE, when present, showed place-fixed behavior. To further analyze these data, group delays (GDs) were computed for derived f1 sweeps for constant f2 frequencies across the entire response area. Delays were based upon nine data points and processed as a running least-squares fit. The resulting GDs were then contour plotted as a function of f2/f1 ratio and f2 frequency. This procedure resulted in cochlear delays of approximately 6 ms at an f2 of 1 kHz, and decreasing to around 1.5 ms at an f2 of 5 kHz. These values compared favorably to other human GDs reported in the literature (e.g., Schoonhoven et al., 2001). Algorithms were also developed so that GDs could be derived for constant f1 with swept f2, or constant ratio trajectories through the data. These procedures clearly illustrated the well-known effects of sweep method on GDs. Average GDs of normal males as compared to normal females were not remarkably different but average GDs of males with NIHL tended to be slightly shorter than the average GDs for normal males.

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O41

Measuring olivocochlear feedback via modulation of otoacoustic emissions: an individualized measurement paradigm

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To date two putative effects of the efferent innervation of the cochlea are discussed: 1. improvement of sound discrimination in background noise, 2. a protective effect against acoustic trauma of the cochlea. Activity of the olivocochlear efferents can be estimated via modulation of active sound emissions of the cochlea (i.e. otoacoustic emissions, OAE) during acoustic stimulation, the so called olivocochlear reflex (OCR).
OCR during contralateral acoustic stimulation was measured in frequencies with distinct non-monotonicity in the level versus frequency function of distortion products of OAE ("DPOAE fine structure dip"), and in frequencies without dip. 121 different primary tone level combinations were used (L1=50-60 dB SPL, L2=35-45 dB SPL, 1 dB steps). The measurement was repeated on another day. The primary tone level had a crucial influence on OCR magnitude. OCR changed by up to 23 dB following a L1-change of only 1 dB. Secondly, OCR-values were generally larger in the dip-frequencies compared to the frequencies with flat fine structure. Both findings can be interpreted in the light of the two-generator model of DPOAE (Heitmann et al. 1998).
In consequence, we suggest to measure OCR preferably in frequencies with distinct dips in DPOAE fine structure. This strategy is proposed to allow for a more targeted search for maximum OCR effects, thus more likely describing the entire range of an individual´s OCR strength. Future studies must show if this approach can contribute to the further clarification of the physiological roles of the cochlear efferent innervation.

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Session VIII: *Cell and Gene Therapy*

O42

Distribution of hematopoietic stem cell-derived cells in mouse cochlea and their possible roles

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Previous investigations have suggested correlation between auditory function and bone marrow (BM) function. However, actual roles of bone marrow-derived cells in auditory systems have not been fully understood. In this paper, we report the distribution of hematopoietic stem cell (HSC)-derived cells in the cochlea and discuss on their roles in cochleae.
HSCs collected from BMs of green fluorescent protein (GFP)-transgenic mice were injected into a tail vein of C57BL/6 mice that had been irradiated. Numerous GFP-positive cells were found in cochleae 3 or 6 months following HSC transplantation. HSC-derived cells were located in the spiral ligament, spiral limb, spiral ganglion and cochlear modiolus, and exhibited the expression of Iba1 or F4/80, a marker for microglias or macrophages. These findings indicate that certain cell populations, which have differentiated into microglias or macrophages, in cochleae are derived from BMs.
We examined alteration in numbers of microglias and macrophages in cochleae in response to BM stimulation. In cochleae treated with systemic application of macrophage-colony stimulating factor (M-CSF), significant increase of microglias/macrophages in the spiral ganglions and cochlear modiolus was observed, which also supports the hypothesis that continuous supply of microglias/macrophages from BMs to cochleae.
We then examined the response of microglias/macrophages in cochleae to local stimulation. Microglias/macrophages in the spiral ligament exhibited a temporal increase after an injection of a culture medium into the posterior semicircular canal, indicating microglia/macrophage systems in cochleae may respond to changes of local circumstances of cochleae.
In conclusion, present findings demonstrate that microglias/macrophages in cochleae are continuously supplied from BMs, and can respond to local stimulation to inner ears, indicating importance of BMs for maintenance of cochlear homeostasis.

O43

The role of bone marrow derived stem cells in inner ear repair

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Hair cell loss in the inner ear leads to hearing impairment and balance dysfunction. Remaining hair cells are unable to reconstitute the degenerated tissue, resulting in permanent functional deterioration. Recent studies suggested that bone marrow (BM) derived stem cells (SC) can contribute to regeneration processes in various degenerated tissues by migration and differentiation, thus promoting structural and functional repair. This high degree of stem cell plasticity prompted us to determine whether BM derived SC could be recruited into the inner ear when an injury is inflicted. We transplanted whole bone marrow cells isolated from C57BL/6 transgenic mice that ubiquitously express enhanced green fluorescent protein (EGFP) under the control of a chicken beta-actin promoter and cytomegalovirus enhancer into lethally irradiated wild C57BL/6 mice. Then, induced inner ear injury by kanamycin and carried out further analysis on the cochlea. Histological analysis performed at 1 and 4 weeks after kanamycin treatment showed a marked infiltration of EGFP+ cells in the deafened cochlea. To examine the potential contribution of the BM derived cells to inner ear regeneration, the cell lineage of the infiltrated cells will be identified, cell differentiation will be traced, and BMSC will be mobilized by treatment of granulocyte colony-stimulating factor (G-CSF) and stem cell factor (SCF) to promote cell differentiation. This work highlights the inner ear intrinsic repair capability of a mammalian model when the inner ear is injured.

O44

Cell transplantation to the auditory nerve and cochlear duct

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We have developed a technique to deliver cells to the inner ear without injuring the membranes that seal the endolymphatic and perilymphatic chambers. The integrity of these membranes is essential for normal hearing, and the technique should significantly reduce surgical trauma during cell transplantation. Embryonic stem cells transplanted at the internal auditory meatal portion of an atrophic auditory nerve migrated extensively along it. Four–five weeks after transplantation, the cells were found not only throughout the auditory nerve, but also in Rosenthal's canal and the scala media, the most distal portion of the auditory nervous system where the hair cells reside. Migration of the transplanted cells was more extensive following damage to the auditory nerve. In the undamaged nerve, migration was more limited, but the cells showed more signs of neuronal differentiation. This highlights an important balance between tissue damage and the potential for repair.

O45

BDNF gene delivery into the mouse cochlea by cell transplantation

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Sensorineural hearing loss is one of the most common disabilities in the industrialized countries, but spontaneous regenerations of the inner ear are not yet possible. Treatment options are currently limited to cochlear implants and hearing aids. Previous studies have indicated the efficacy of gene therapy for the treatment of the inner ear. However, various problems are still remained before clinical application. For instance, a use of viral vectors involves the risk of virus toxicity and uncontrolled gene expression despite its high efficiency for transduction and expression.
One of possible strategies to resolve this problem is ex vivo gene therapy using non-virus vectors. In this study, we examined the ex vivo gene delivery to the mouse cochlea using gene-modified cells with non-virus vector.
Mouse brain-derived neurotrophic factor (BDNF) gene was used as a gene to delivery into the cochlea, because BDNF has various biological effects including trophic supports in neural systems. NIH3T3 cells were transduced the BDNF gene using lipofection and then transplanted into the mouse inner ear through the posterior semicircular canal. Immunohistochemistry and Western blots demonstrated the survival of grafted cells in the cochlea for 4 weeks after transplantation. No significant hearing loss was induced by the transplantation procedure. A BDNF-specific enzyme-linked immunosorbent assay (ELISA) revealed a significant increase in BDNF production in the inner ear following transplantation of gene-engineered cells. These findings suggest that ex vivo gene therapy may be feasible for the treatment of the inner ear disorders through the local and sustained delivery of therapeutic molecules to the cochlea.

O46

Time course of hair cell death in the cochlea of connexin30 knockout mice and its complete rescue by augmented connexin26 protein expression

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Mutations in connexin26 (Cx26) and Cx30 genes are most common genetic defects responsible for about half of inherited prelingual non-syndromic deafness cases. The most commonly found human Cx mutations in either Cx26 (e.g., 35delG) or Cx30 (e.g., e.g., GJB6-D13S1830) effectively eliminate gene expression. Targeted deletion of either Cx26 or Cx30 coding sequence in mice results in deafness.
We characterized the time course of apoptotic cell death in the cochlea of Cx30-/- mice. Results showed that at postnatal day 18 (P18) to P21, most inner hair cells (HCs) and outer HCs in Cx30-/- mice were intact except those in the basal turn. However, the hearing losses across a frequency range of 4-32 kHz were >30dB. A near complete loss of HCs didn't happen until two months after birth. Microarray studies identified the activation of many apoptosis genes before a complete HC death, especially those involved in the mitochondria apoptosis pathway that releases cytochrome C into cytoplasma.
Cx26 and Cx30 are the two major Cx isoforms found in the cochlea and they co-assemble to form heteromeric GJs. Such molecular arrangement implies that homomeric GJs would remain in the cochlea if one of the co-assembly partners is mutated resulting in null expression. To test whether insufficient number of functional GJ channels causes deafness in Cx30-/- mice, we generated mice in which extra copies of the Cx26 gene were transgenically expressed from a modified bacterial artificial chromosome (BAC) in Cx30-/- background. In the absence of Cx30 gene, Cx26 expressed from extra alleles prevented hair cell death and completely restored hearing sensitivity of deaf Cx30-/- mice. The results indicated that heteromeric GJs were not essential for normal hearing, and suggested that up-regulation of Cx26 or slowing down its protein degradation might be a therapeutic strategy to prevent and treat deafness caused by Cx30 mutations.

O47

Over-expression of XIAP delays the development of presbycusis in C57Bl/6 mice

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Apoptosis has been implicated in several neurodegenerative disorders including age-related hearing loss, also known as presbycusis. C57BL/6J mice can be used as an animal model for early development of presbycusis. In the present study, we evaluated whether over-expression of X-linked Inhibitor of Apoptosis Protein (XIAP) prevented or delayed the development of presbycusis. Transgenic mice, on a C57Bl/6 genetic background, were used that ubiquitously over-express XIAP using a human *xiap* transgene (ubXIAP). It was determined that this transgene expression did not change with age.

The hearing status and cochlear pathology were compared between the transgenic ubXIAP mice and their wild type (WT) littermates. Hearing status was evaluated using frequency-specific auditory brainstem responses (ABR). The degenerative pathology of the cochleae was evaluated by cochleogram.

Our results demonstrate that WT mice began to show signs of presbycusis at two months of age, as verified by comparing the ABR with CBA mice. The development of hearing loss was slower and significantly smaller in ubXIAP than WT controls up to 4 months of age. Thereafter, the development of hearing loss appeared to be similar between the groups; ubXIAP mice appeared to have better hearing by 5-30 dB across the frequency range tested. Consistent with these results, cochleograms showed that there was a smaller amount of hair cell loss in the ubXIAP than WT mice. Taken together, these results suggest that XIAP overexpression can delay or reduce age-related hearing loss and cell death in the cochlea.

Session IX: *Ionic Signalling*

O48

The mechanotransducer channels of mammalian cochlear hair cells

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Hair cells detect sound stimuli by vibration of their hair bundles which activates mechanotransducer (MT) channels probably by tensioning tip links between stereocilia in adjacent ranks. The MT channel is one of the few ion channels not to have been identified at a molecular level so it is important to catalog its properties in situ for comparison with cloned variants. We have therefore measured MT currents and single-channel attributes for inner (IHC) and outer (OHC) hair cells in isolated rat cochleas at positions with different characteristic frequencies (CF). The MT channel is a calcium selective channel, activated by hair bundle displacements of a few hundred nanometers and displaying rapid activation followed by sub-millisecond adaptation. Calcium influx through the channel is important for triggering adaptation to optimize transducer sensitivity. For OHCs, the peak current size increases with CF but IHCs show little equivalent tonotopic variation. Also the time constant of fast adaptation decreases with CF for OHCs but is invariant for IHCs. To understand the tonotopic variation we isolated single MT channels after brief exposure to BAPTA. MT channels from IHCs are deduced to have a conductance (in 0.02 mM calcium) of 260 pS, but those from OHCs depend on CF, increasing from 130 pS at the apex to 260 pS at the base. Estimates of the number of tip links from scanning electron micrographs indicate a small increase with CF for OHCs but none for IHCs. The combination of MT channel conductance and tip link number can account for the change in the OHC macroscopic current with CF and its invariance for IHCs. For both cells we calculate 1 to 2 channels per tip link. The larger channel size in basal OHCs increases sensitivity and, by allowing larger calcium influx, speeds up adaptation. Our results imply the channel has unusual properties possessed by few candidates. Moreover, variation along the tonotopic axis suggests occurrence of multiple channel subunits.

O49

Local Ca^{2+} as indicator of the location of mechanoelectrical transduction channels

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Mechanoelectrical transduction (MET) converts mechanical vibration of stereocilia (SC) into electrical signals in the hair cells. Entry of Ca^{2+} ions through MET channels into the SC, and their intracellular regulation, is crucial for MET. The goal of the present study was to localize MET channels in the hair bundle of freshly isolated outer hair cells (OHCs) from the adult mammalian cochlea.

OHCs were mechanically isolated from the adult guinea-pig cochlea. Ca^{2+} transients were evoked by fluid-jet stimuli. To facilitate Ca^{2+} entry into the hair bundle, the Ca^{2+} concentration in the fluid-jet solution was 4 mM (extracellular 100 µM). Ca^{2+} concentration changes were monitored using the acetoxymethyl ester form of the fluo-3 dye and confocal laser-scanning microscopy.

The stimulation evoked a fast increase of the intracellular Ca^{2+} concentration in the hair bundle. Application of the MET channel blockers (DHSM, 100 µM) did not change the amplitude of the Ca^{2+} transients, but the onset time constant was significantly increased by the drug at the tip link region by a factor of about 8. Linescan transects were made of the tip link region of the hair bundle. Analysing each half micrometer sections of the linescan images, the onset time constant was the smallest (30±2 ms) at a distance of 1.75 µm from the cuticular plate (CP). This region is at the top of the middle row of SC. Moving closer or away from the CP, the speed of the Ca^{2+} transients became significantly slower (56±4 ms at 0.25 um and 54±5 ms at 2.75 µm, respectively). The time constant in the longest SC was around 300 ms, which was independent of distance from the CP. MET channel blockers like DHSM or Gd^{3+} resulted in a prominent reduction in the rate of Ca^{2+} elevation at the top of the shorter stereocilia in the region of tip link.

These results suggest that MET channels are located only at the lower end of the tip link.

O50

Long-range signalling by calcium waves in the organ of Corti

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Studies in different types of cell cultures have shown that damage and mechanical stimulation can activate changes in intracellular free calcium concentration and elicit intercellular calcium waves. Thus an attractive hypothesis is that calcium transients propagating as a wave through support cells in the organ of Corti may constitute a fundamental mechanism to signal the occurrence of hair cell damage. The mechanism we have characterized exhibits nanomolar sensitivity to extracellular ATP, a key neuromodulator of visual and auditory sensory epithelia. In the rat cochlea, pharmacological dissection indicates that ATP, acting through a highly sensitive purinergic / IP3-mediated signaling pathway with (little or) no involvement of ryanodine receptors, is the principal paracrine mediator implicated in the propagation of calcium waves though Hensen's and Claudius supporting cells. Indeed, calcium waves elicited under highly reproducible conditions by carefully controlling dose (1 µM) and timing of focal ATP application (200 ms), extended over radial distance greater than 160 µm from the source, identical to those activated by damaging single hair cells. Measurement of sensitivity to UTP and other purinergic agonists implicate P2Y2 and P2Y4 as the main P2Y receptor isoforms involved in these responses. Injection of IP3 in supporting cells of the rat organ of Corti, which are interconnected by gap junctions formed by connexin 26 and connexin 30, ensues in a regenerative wave of calcium throughout the tissue. Altogether, these results indicate that intercellular calcium waves are a robust phenomenon that confers a significant ability for cell-cell communication in the mammalian cochlea. Further ongoing research will reveal the roles that such calcium waves play in the inner ear.

O51

Ultrastructural localization of large calcium activated potassium channels in the rat cochlea

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Large calcium activated potassium channels (BK channels) are gated by both membrane depolarization and an increase in cytosolic calcium. In lower vertebrate hair cells, BK channels are localized in the pre-synaptic zones near the calcium channels that are believed to regulate neurotransmitter release. These channels have also been found in mammalian inner (IHCs) and outer hair cells (OHCs), but their precise ultrastructural distribution has not been reported. Postembedding immugold labelling has been used to localize these channels. Cochleas from post-hearing Sprague Dawley rats were fixed in 4% paraformaldehyde and 0.1% glutaraldehyde and embedded in LR White resin. Ultrathin sections were immunogold labelled using a polyclonal anti-BKCa channel antibody (Alomone Labs) and a secondary antibody conjugated to gold particles. In hair cells, labelling was found on the basolateral plasma membrane, mitochondria, cuticular plate and stereociliary membrane. In IHCs, plasma membrane labelling was concentrated in patches below the cuticular plate as well as being distributed more evenly elsewhere along the membrane; in OHCs, membrane labelling was sparser and more evenly spaced. Labelling also occurred on mitochondria of cochlear nerve fibres and supporting cells and on the plasma membrane of OHC efferents and several types of supporting cells, especially associated with tight junctions in the reticular lamina. Thus, we have found ultrastructural labelling for BK channels in locations reported light microscopically by other groups (e.g., Pyott et al., 2004, J Neurosci 24, 9469- 9474) but this method allows now to determine quantitatively its distribution. It is also interesting to note the mitochondrial labelling as it has recently been reported that BK channels occur in the inner mitochondrial leaflet of rat brain (Douglas et al., 2006, Neurosci 139, 1249-1261). These channels may therefore have a wider range of functions in the cochlea than has previously been supposed.

O52

Acid sensing ionic channels in the vestibular endorgans

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Acid-sensing ionic channels (ASICs) are members of the epithelial sodium channel/degenerin (ENaC/DEG) super family. ASICs are widely distributed in the central and peripheral nervous system. They participate in synaptic transmission, pain perception, and the mechanoreception in peripheral tissues. Our objective was to characterize proton-gated currents mediated by ASICs and to determine their immunolocation in the rat vestibular periphery. Whole-cell Voltage-clamp of cultured afferent neurons from P7 to P10 rats showed a proton-gated current with rapid activation and complete desensitization, which was carried almost exclusively by sodium ions. The current response to protons had a pH0.5 = 6.2. This current was reversibly decreased by amiloride, gadolinium, lead, acetylsalicylic acid, and enhanced by FMRFamide and zinc, and negatively modulated by raising the extracellular calcium concentration. Functional expression of the current was correlated with smaller capacitance neurons. Immunoreactivity to ASIC1a and ASIC2a subunits was found in small vestibular ganglion neurons and afferent fibers that run throughout the macula utricle and crista stroma. ASIC2b, ASIC3, and ASIC4 were expressed to a lesser degree in vestibular ganglion neurons (ASIC1b subunit was not detected in the vestibular endorgans). No proton gated currents or ASIC immunoreactivity were found in hair cells. Acidification of the extracellular pH generated action potentials in cultured vestibular neurons, and FMFRamide was shown to increase the resting discharge of the afferent neurons recorded in the isolated vestibule of the rat vestibule. Our results indicate that proton-gated current is carried through ASICs, whose expression is segregated to small size afferent neurons and that ionic current activated by protons contributes to shape the vestibular afferent neurons response to its synaptic input.

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IEB-2006 WORKSHOP

Poster Presentations

Abstracts

Normal structure and function

P1-P4

P1

Excitability properties of rat vestibulocerebellum interneurons

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Information concerning head position and rotation is detected by vestibular hair cells, and relayed to the vestibular nuclei (VN) and to the cerebellum via primary vestibular neurons (PVNs). Unipolar brush cells (UBCs) and granule cells (GCs) are interneurons of the granular layer of the vestibulocerebellum that receive signals from PVNs and VN. We have investigated the electrophysiological properties of UBCs and GCs located in the vestibulocerebellum lobi I (lingula) and X (nodulus), by combining the patch-clamp technique in whole-cell configuration with acute rat cerebellar slices (postnatal day 17-23). UBCs (n=13) showed a mean membrane capacitance (Cm) of 9.8 ± 3.2 pF, a mean input resistance (Rm) of 0.81 ± 0.41 GOhm, and a mean resting potential (Vr) of –54.4 ± 7.5 mV. GCs (n = 21) had a mean Cm of 3.3 ± 0.9 pF, a mean Rm of 3.3 ± 0.5 GOhm, and a mean Vr of –55.9 ± 8.7 mV. The voltage responses of UBCs and GCs was significantly different: when depolarised from –70 mV by current steps large enough to reach threshold, UBCs showed a transient (phasic) discharge of action potentials lasting less than 50 ms, whereas GCs showed a sustained (tonic) discharge of action potentials lasting for the whole current step duration (1000 ms). These differences were associated with different patterns of ionic currents recorded in voltage-clamp mode: an initial characterisation revealed that UBCs express a slow inward rectifying current (Ih), with or without an anomalous K^+ rectifying current (IK1). Conversely, all GCs expressed IK1, whereas none expressed Ih.
Since the afferent activity of PVNs mainly differs in response dynamics, with purely phasic and tonic discharges at the two extremes of a broad dynamics range, present results suggest that UBCs intrinsic properties would match phasic PVNs, whereas GCs would match tonic PVNs.

P2

Morphometry of the reticular lamina (SEM and LSM investigations on guinea pig cochlea)

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Geometrical data of the Reticular Lamina (RL) is important for models of Cochlea mechanics. Most of the existing data about RL structures was obtained by scanning electron microscopy (SEM) examinations. This method demands complete dehydration, which induces shrinking of cellular structures. The aim of our study was to compare the traditional SEM data with data from confocal laser scanning microscopy (LSM). The LSM was taken as a reference, since the tissue shrinkage due to the preparation process is presumed to be insignificant (Edge 1998).
The morphometry of the RL as well as the stereocilia bundles positions in all rows of hair cells along the Guinea Pig cochlea were examined by means of SEM and LSM (Philips XL30 ESENS and Zeiss LSM 510 META). For SEM investigations the specimen was dehydrated using a conventional preparation method and sputtered with an extremely thin layer of gold (~10 nm). The dimensions of RL and the position of the stereocilia bundles were measured with up to 50,000 times magnification. For LSM investigations the specimen was fixed with 4% neutral buffered formaldehyde solution. Afterwards it was stained with fluorescent dye Phalloidin-TRITC in standard histochemistry protocol. The RL and the stereocilia bundles at the same regions (as for SEM) of the Basilar membrane were scanned with up to 1,000 times magnification and analyzed with Zeiss LSM Image Browser 4.0.
The study showed that shrinkage after conventional preparation for SEM can be up to 300 % compared to LSM data. Dimensions of the whole RL were more sensitive to this process than the shape of the stereocilia bundles. The distance between individual bundles changes proportional to the common shrinkage factor of the RL.
Conclusions: For investigations on cochlea morphometry the LSM provides more precise data concerning the genuine tissue structure. However SEM provides better resolution and visualizes finer structures. When using SEM data the shrinkage factor must be consider.

P3

A novel method for distinguishing between Type I and Type II spiral ganglion neuron input into the mouse cochlear nucleus

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The primary auditory neurons are comprised of Type I and Type II spiral ganglion neurons (SGNI and SGNII) that are distinguishable by their peripheral sensory targets. In contrast, discrimination between central synaptic targets of SGNI and SGNII is more difficult because SGNII represent <10% of the total SGN. This study aims to resolve and compare these central projections in mice by immunolabelling for peripherin, an intermediate filament protein that is exclusively expressed by SGNII, in conjunction with the application of tetramethylrhodamine dextran (TMRD), a neuronal tracing dye that we have found is only transported by SGNI axons.

Under anaesthesia, the SGN axons within the modiolus of 21-day old mice were severed and TMRD applied. Animals were subsequently killed, fixed, and counterstained using peripherin antisera with an Alexa488-conjugated secondary antibody. Cryosections of the brainstem were imaged using confocal microscopy.

TMRD-labelled fibres projected to the anteroventral (AVCN), posteroventral and dorsal cochlear nuclei (DCN), representing the central projections of the SGNI. The large calciform terminations of the SGNI, the endbulbs of Held, were observed in the AVCN. TMRD-labelled neurites did not show peripherin immunolabelling, indicating that SGNII did not transport the TMRD dye. Peripherin immunolabelled fibres sparsely innervated the cochlear nucleus and a distinction could be made between two types of fibres, where those that innervated the AVCN and the DCN were of a smaller diameter than those that innervate the granule cell region, which surrounds these two nuclei.

This study describes a method to discriminate between the adjacent central projections of SGNI and SGNII to the cochlear nuclei. This technique will permit in vitro recordings of second order neurons on which labelled neurites synapse.

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P4

Two classes of outer hair cells along the tonotopic axis of the cochlea

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The molecular basis of high versus low frequency hearing loss and the differences in the sensitivity of outer hair cells depending on their cochlear localization is currently not understood. Here we demonstrate the existence of two different outer hair cell phenotypes along the cochlear axis. Outer hair cells in low frequency regions exhibit early sensitivity for loss of the Cav1.3 Ca^{2+} channel, while high frequency regions display progressive susceptibility for loss of the Ca^{2+}-activated large conductance K^{+} (BK) channel. Despite deafness, young Cav1.3-deficient mice displayed distortion-product otoacoustic emissions (DPOAEs) indicating functional outer hair cells in the higher frequency range of the cochlea. Considering that DPOAEs despite profound hearing loss are also found in the human deafness syndrome DFNB9 caused by mutations in the synaptic vesicle protein otoferlin, we tested the expression of otoferlin in outer hair cells. Surprisingly, otoferlin showed a distinct tonotopic expression pattern at both mRNA and protein level revealing Cav1.3 deletion-sensitive, otoferlin-expressing outer hair cells in the low frequency range being clearly separated from BK deletion-sensitive, otoferlin-negative outer hair cells in the high frequency range. In addition, BK deletion led to a higher noise vulnerability in low frequency regions, which are normally unaffected by the BK deletion alone, indicating that BK currents are involved in survival mechanisms of outer hair cells under noise conditions.
Our findings suggest new mechanisms and candidate genes for explaining high and low frequency hearing loss.

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Endolymph Homeostasis

P5-P7

P5

Functional expressions of KCNQ1/KCNE1 K^+ channel and P2Y4 receptor

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This study was conducted to investigate the functional expressions of KCNQ1/KCNE1 K^+ channel and P2Y4 receptor in developing rat strial marginal cells using a voltage-sensitive vibrating probe. Chromanol 293B, a blocker of KCNQ1/KCNE1 K^+ channel, inhibited short-circuit currents (Isc) from postnatal day 1 (P1) to P21. Similarly, Isc were found to be decreased by uridine 5'-triphosphate at all ages. The ineffectiveness of suramin and pyridoxalphosphate-6-azophenyl-2',4'-disulfonic acid to block the action of uridine 5'-triphosphate indicated that the apical P2Y receptor is P2Y4. The authors conclude that KCNQ1/KCNE1 K^+ channels are expressed in developing strial marginal cells, and that the coincidence of P2Y4 receptors might provide a protective dampening of K^+ influx through hair cell in response to intense noise exposure.

P6

Electrophysiological recordings of the utricular endolymphatic potassium concentration

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The high K^+ concentration within the endolymph and the trans-epithelial voltage across sensory epithelia are necessary for hair cells to transduce balance stimuli. Indeed, both provide with the driving force for K^+ to flow through stereocilia transducer channels. Alteration in the endolymph ion homeostasis would disrupt the driving force leading to dysfunctions like some vertigo. With the aim of understanding the regulation of the K^+ endolymph concentration, we developed an utricular organotypic model able to recreate the endolymph compartment (cyst). Here, we further characterize this model by determining K^+ accumulation features within the neo-formed cyst. Utricles dissected from newborn and neonate mice and rats were grown in 3D matrix. After 2-4 days in vitro (div), utricles regenerate endolymph cysts easily recognizable under a dissecting microscope. This regenerating potential was assessed by determining the rate of cyst formation during the postnatal period. This rate is higher in mouse tissues than in rat ones. Indeed, 60-80 % of cultured mouse utricles rebuild cysts when taken from 0 to 4-day-old animals, while 40-55 % of cultured rat utricles restore vesicles during the same developmental period. Regeneration ability disappears by postnatal day 8 in both species. Next, K^+ accumulation was demonstrated within cysts by means of K^+ selective electrodes that record K^+ mediated currents. K^+ concentrations are derived via calibration curves. Rat cyst K+ concentration increases from div 6 to 11 where it peaks at 102 ± 6 mM and decreases afterwards. In mouse cyst, K^+ accumulates from div 4 to 8 where it reaches a plateau level around 47 mM that lasts for 7 div. These data confirm that rat utricles rebuild endolymph cysts in vitro, where K^+ concentrates, and extend these findings to mouse utricle. This model can be maintained in vitro for several days, hence being a useful tool for investigating the regulation of endolymph ion homeostasis.

P7

Expression of AQP2, vasopressin type 2 receptor, TRPV1 and TRPV4 in the human endolymphatic sac

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Objective: To localize aquaporin-2 (AQP2), vasopressin type 2 receptor (V2-R) and transient receptor potential channel vanilloid subfamily-1, -4 (TRPV1, TRPV4) in the human endolymphatic sac.
Material and Methods: The human endolymphatic sac was harvested during the removal of acoustic neurinoma with translabyrinthine approach. The samples were immediately fixed in 4% paraformaldehyde and embedded in OCT compound; immunohistochemistry were performed with AQP2, V2-R, TRPV1 and TRPV4 polyclonal antibodies.
Results: AQP2, V2-R and TRPV4 proteins were detected in the epithelium of the endolymphatic sac. TRPV1 was not clearly expressed in the epithelium of the endolymphatic sac, but in the vascular endothelial cells of the connective tissue of the endolymphatic sac.
Conclusions: AQP2, V2-R and TRPV4 were clearly expressed in the endolymphatic sac. The same characteristic distribution of water and ion channels is seen in the kidney, where a great amount of fluid is filtrated and resorbed. The endolymphatic sac probably plays an active role in the homeostasis of endolymph.

Micromechanics

P8-P13

P8

Prestin and a functional tectorial membrane are not required to generate electrically evoked otoacoustic emissions

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Electrically evoked otoacoustic emissions (EEOAEs) are sound emissions from the inner ear generated when alternating current (AC) is injected into the cochlea. EEOAEs can be easily measured in the ear canal and are usually attributed to the motile responses of outer hair cells. Multi-component analysis reveals that EEOAEs consist of a short (SDC) and a long delay (LDC) component. The SDC is very robust and a residue can still be measured after almost complete elimination of outer hair cells. Therefore, the function of outer hair cells as the sole generators of the SDC is questionable. Alternatively, a hypothetical tectorial membrane (TM) electromotility could contribute to the generation of EEOAEs. We are aiming to answer two questions:
1. Are EEOAEs produced by prestin-mediated somatic motility?
2. What is the contribution of the tectorial membrane to the generation of EEOAEs?
To address these questions we used prestin knockout mice and Tecta delta ENT/ delta ENT mice, in which the TM is completely detached from the organ of Corti and non-functional. We applied AC currents to the round window while recording sound emissions from the ear canal. Prestin knockout mice and Tecta delta ENT/ delta ENT mice both produced EEOAEs, albeit at a lower sound pressure in comparison to wild type animals. Typical for extracochlear round window stimulation, the frequency response is broad and EEOAEs could be evoked at all frequencies tested (10-80 kHz) at moderate stimulation intensities (up to 50 microamperes). Multi-component analysis of the real parts of the recorded sound spectra revealed SDCs and LDCs in all mice, with prestin knockout mice displaying a less prominent LDC in comparison to their wild type and the Tecta mice having delay spectra similar to their wild type. Our results support the suggestion previously made by others that a non-hair cell structure is possibly involved in the generation of EEOAEs, but a major contribution from the TM can be ruled out.

P9

Basilar membrane response to electrical stimulation in mice with and without the tectorial membrane load

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The large dynamic range, sensitivity and frequency selectivity of the cochlea are due to amplification of responses at low level sounds and compression at high levels by the outer hair cells (OHC). Two mechanisms have been proposed as a basis for cochlear amplification: active bundle movement and OHC motility, but their relative contributions are unclear in the mammalian cochlea. We have stimulated mice cochleae with AC current using a metal electrode on the round window and an Ag/AgCl pellet on the neck. Currents of different frequencies and levels were injected and a laser interferometer was focused on the high frequency region of the basilar membrane (BM) to obtain tuning curves of the mechanical response. Experiments were conducted on wild type and Tecta mice. Tecta mice contain a mutation of a tectorial membrane (TM) glycoprotein, alpha-tectorin. In homozygous mice, lacking alpha-tectorin, the TM is detached from the organ of Corti. Heterozygous mice have a normal TM (Legan et al., 2000). The electrically evoked BM tuning of wild type and heterozygous mice cochleae resemble acoustic tuning previously reported for the same region, with a tip and a second threshold minimum about half an octave below it. BM responses to electrical stimulation from homozygous, heterozygous and wild type mice are similar in tuning and sensitivity. This differs from previous acoustic tuning curves where homozygous mice were less sensitive and without the second peak. Both peaks are associated with cochlear amplification at low levels and compression at high levels. BM displacements in response to electrical stimulation are blocked by salicylate applied via the round window membrane. Sharp, sensitive frequency tuning can, therefore, be electrically elicited from the cochleae of Tecta mice in which somatic motility is present but the TM load required for any possible hair bundle force to be fed back into the system is missing.

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P10

Influence of the tectorial membrane resonance on the cochlear acoustical and neural responses

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Mechanically coupled cochlear structures are likely to form a resonator with a few degrees of freedom. Consequently one can expect complex, frequency dependent relative movements between these structures, for example between the tectorial membrane (TM) and reticular lamina that excites the cochlear receptors. This excitation should be minimal at the frequency of a hypothetical TM resonance. In this work simultaneous masking neural tuning curves and distortion product otoacoustic emissions were recorded from the same preparations. The position of the low-frequency minima in the tuning curves, frequency dependence of the emission band-pass structure and level-dependent phase inversion were compared to determine if their generation is due to the same phenomenon, e.g. due to the tectorial membrane resonance. The notch in the masking tuning curves and the phase inversion of the emission growth functions at the auditory thresholds are both situated 0.5 octaves below the probe frequency and the high frequency primary, respectively, and show similar dependence on these frequencies. However, the emission band-pass structure is likely to be due to a combination of different mechanisms suggesting that the TM resonance is not the main factor contributing to its formation, especially at the primary frequencies above 10 kHz. For primary frequencies above 10 kHz and varying primary frequency ratio, the amplitude of the emission shows several local maxima at frequencies separated by half octave intervals. Frequencies of the maxima are determined by the intrinsic properties of the guinea pig cochlea because they do not depend on the primary frequencies and are the same for emissions of different orders. These local maxima possibly reflect the formation of standing waves in the cochlea, which effects the emission production either at the primary or at the emission characteristic frequency places.

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P11

The tectorial membrane: trading frequency selectivity and sensitivity in the mammalian cochlea

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Frequency tuning in the mammalian cochlea is determined by the mechanical properties of the basilar membrane (BM) and its interactions with the outer hair cells (OHCs) and the overlying tectorial membrane (TM), with most interventions and injuries causing a loss of tuning and deafness. Here we show that the loss of the glycoprotein Tectb results in a significant sharpening of BM and neural frequency tuning. Sharpened frequency tuning is associated with a 5-10 dB loss in sensitivity at the tips of the tuning curves. There is an overall decrease in cochlear sensitivity in apical regions of the cochlea sensitive to frequencies below 20 kHz. Extracellular receptor potentials recorded from the OHCs in the high frequency region of the cochlea are normal. Distortion product otoacoustic emissions are generated only in the high frequency regions of the cochlea at low sound levels, spreading to lower frequencies with increasing sound level. The attenuation in the spread of excitation along the length of the cochlea is strongly associated with the loss of the striated sheet matrix from the TM whereas all other structures within the cochlear partition appear normal. It might be deduced, therefore, that the striated sheet matrix is essential in mechanical coupling of the TM elements along its longitudinal axis. Loss of the matrix causes a reduction in the ability of OHCs in adjacent frequency locations along the length of the cochlea to influence each other via their attachment to the TM and, hence, to respond in synchrony in effectively amplifying the cochlear vibrations. We conclude that the TM has a distinctive role in determining frequency resolution and the spread of excitation along the length of the cochlea so that the observed cochlear sensitivity and frequency resolution is achieved through a trade-off based on the number of OHCs contributing to the amplification of a single frequency place in the cochlea.

P12

The effects of cholesterol on outer hair cell motility

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Several lines of evidence indicate that hearing in mammals owes its preciseness to amplification by the electromotility of outer hair cells (OHCs). The effect of increased blood cholesterol level on hearing in human beings and its mechanism are still controversial. Previous study demonstrates that exogenous cholesterol is taken up within the plasma membrane (PM) and reduces its flexibility and fluidity, implying that increased cholesterol in the PM can affect hearing via acting on the OHC directly.
Here, we examined the effects of cholesterol on isolated OHC function. Atomic force microscopy revealed that application of cholesterol remarkably increased the axial stiffness of OHCs from 1.11 to 4.74mN/m. To measure OHC axial length change, OHC moving images were analyzed with DIAS software, which detected the cell edges automatically. Analysis of the cell length change demonstrated that both the prestin-dependent “fast” electromotility and the prestin-independent “slow” motile response were reduced by cholesterol. Cholesterol compressed cell length change induced by voltage change in load-free OHCs by 78 %, although the charge movement induced by voltage change was not affected. This result suggests that cholesterol reduces efficiency of inducing cell length change for given charge movement while the activity of the prestin itself is not affected by cholesterol. Cell length change caused by extracellular osmolarity alteration (50mOsm) was significantly reduced by cholesterol. Effects of modification of the extracellular osmolarity on the peak CM voltage shift, presumably resulting from the change in tension between the motors, were attenuated by cholesterol, indicating that cholesterol could reduce the increase of membrane tension caused by turgor pressure elevation. These results suggest that cholesterol may change the characteristic frequency and operating range of the OHC, resulting in reduction of cochlear amplification and hearing acuity.

P13

Electrogenic chloride and bicarbonate transport by prestin (SLC26A5) in expression systems

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Prestin, the motor protein of outer hair cells, is a member of the SLC26 gene family which encodes highly versatile and ubiquitous proteins capable of transporting a wide variety of monovalent and divalent anions. Prestin shares overall homology with the nine other family members but has instead been described as an incomplete transporter, whose role in outer hair cell electromotility is compromised in the absence of intracellular anions (Cl^- and $HCO3^-$). Previous experiments in outer hair cells where both these anions were replaced extracellularly by gluconate and Hepes respectively, suggest that prestin may indeed behave as an electrogenic Cl^- /$HCO3^-$ transporter. To test this hypothesis we carried out functional studies in two different expression systems, CHO and HEK293 cells transfected with rat prestin tagged with GFP. Nonlinear capacitance indicated transfection levels of approximately 2.5 x 10^5 prestin per cell (whereas OHCs express levels at least 10x higher). Whole cell currents were recorded from HEK cells using glass micropipettes filled with a 140 mM Cl^- intracellular solution buffered with 20 mM $HCO3^-$. The change from a $HCO3^-$ to a hepes buffered medium in the presence of low extracellular Cl^- resulted in a 35 pA decrease in the mean outward current of transfected vs untransfected cells. This represents a net electrogenic transport rate of ~850 ions/sec per prestin carried by $HCO3^-$. In a different set of experiments, intracellular pH changes were monitored in BCECF AM-loaded CHO cells with and without prestin. The recovery from intracellular acidification in cells exposed to a Cl^-free bicarbonate buffered solution was faster for transfected cells. The imaging data therefore also supports the proposal that prestin provides a bicarbonate loading mechanism and may therefore, even though of low efficiency, be the main regulator of pHi in outer hair cells.

Ionic Signalling

P14-P17

P14

Mechanisms of calcium homeostasis in cochlear hair cells

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Hair cells use Ca^{2+} entering through channels of stereocilia and basolateral membrane to regulate mechanoelectrical transduction and to mediate synaptic transmission. Here we studied how inner and outer hair cells (IHCs and OHCs) of hearing mice regulate their cytosolic calcium concentration ($[Ca^{2+}]i$) after basolateral or apical Ca^{2+} influx. Increments of cytosolic $[Ca^{2+}]$ in IHCs were induced by voltage-gated Ca^{2+} influx in patch-clamp experiments and monitored by fura-2 photometry. Ca^{2+} influx through transduction channels into OHCs was elicited by fluid jet deflection of hair bundles. The resulting increase $[Ca^{2+}]i$ was measured in the apical compartment of fura-2AM loaded OHCs using CCD-imaging. In order to identify the major clearance mechanisms for cytosolic Ca^{2+} in IHCs and OHCs we examined the effects of pharmacological inhibition of plasma membrane and endoplasmic Ca^{2+} ATPases, Na^{+}/ Ca^{2+} exchanger and mitochondrial Ca^{2+} uptake. In addition, we studied Ca^{2+} clearance in IHCs and OHCs of mouse mutants (deafwaddler2j, dfw2j) lacking the plasma membrane Ca^{2+} ATPase 2 (PMCA2).
Most of the incoming Ca^{2+} was extruded by PMCAs in both OHCs and IHCs as suggested by the effects of the PMCA inhibitor carboxeosin and increased extracellular pH. Clearance of stereociliar Ca^{2+} influx in OHCs was mainly mediated by PMCA2. OHCs of mice homozygote and heterozygote for the dfw2j mutation showed an elevated resting $[Ca^{2+}]i$ and a prolonged $[Ca^{2+}]i$ decay after mechanoelectrical transduction. In contrast, we did not observe any obvious changes in the clearance of basolateral Ca^{2+} influx in PMCA2-deficient IHCs indicating that extrusion can be supported by other PMCA isoforms. In addition, we show that $[Ca^{2+}]i$ homeostasis of IHCs involves Na^{+}/ Ca^{2+} exchanger and mitochondrial Ca^{2+} uptake, whereas endoplasmic Ca^{2+} pumps are not. We postulate that the increased resting $[Ca^{2+}]$ of OHCs first causes impaired mechanoelectrical transduction and finally induces cell death.

P15

Calcium currents in mouse inner hair cells are inhibited by phenylalkylamines and benzothiazepines

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Calcium currents (ICa) in inner hair cells (IHCs), carried mainly by the CaV1.3 subtype of L-type voltage gated calcium channels (Platzer et al., Cell, 2000), are prominently blocked by three structurally different drug classes dihydropyridines (DHPs), phenylalkylamines (PAAs) and benzothiazepines (BTZs). Lower sensitivity of the CaV1.3 calcium current towards DHPs compared to the CaV1.2 current was reported (Platzer et al., Cell, 2000; Michna et al., J. Physiol., 2003). We investigated effects of PAAs (verapamil and gallopamil) and BTZs (diltiazem) on ICa through CaV1.3 channels in mouse IHCs. Whole-cell ICa was measured using the patch-clamp method in IHCs aged P3-P7 with 5 mM Ca^{2+} as charge carrier. The PAAs verapamil and gallopamil and the BTZ diltiazem blocked approximately 90% of ICa at a concentration of 3 mM. This block was largely reversible. Dose-response curves for PAAs revealed IC50 of 199±19 microM for verapamil and 466±151 microM for gallopamil and Hill coefficients of 0.65±0.02 and 0.84±0.2, respectively. Diltiazem blocked ICa with IC50 of 326±67 microM and a Hill coefficient of 0.98±0.16. In conclusion, PAAs and BTZs blocked the L-type Ca^{2+} currents in mouse IHCs in a concentration-dependent manner, which was largely reversible. Blocking concentrations were in the same range as those required for block of ICa in turtle hair cells but Hill coefficients were smaller (Schnee and Ricci, J. Physiol., 2003). In contrast to the action of DHPs, concentrations of PAAs needed to block CaV1.3 currents in IHCs were in the same range as those for CaV1.2 currents (Lacinova et al., J. Pharmacol. Exp. Ther. 1995). BTZ concentrations required for CaV1.3 current inhibition were 500 times higher compared to those for CaV1.2 currents (Kurokawa et al., Mol. Pharmacol, 1997).

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P16

BK channels regulation in rat inner hair cells

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Cochlear inner hair cells (IHCs) act as primary sensory receptors in the auditory pathway, transforming sound-induced mechanical stimuli into a graded receptor potential (RP). In response to depolarization, the cell releases excitatory neurotransmitter onto afferent neuronal fibers. To perform high frequency transduction, IHCs display large outwardly rectifying currents thought to shorten the membrane time constant. IHCs display a complex set of K^+ currents: IKf, IKs and IKn carried by BK, Kv and KCNQ channels, respectively, that can be pharmacologically dissected. A recent study (Oliver et al., 2006) highlights the essential role of BK channels in shaping the RP. BK channels are composed of a tetramer of the pore-forming alpha subunit and accessory beta subunits well known to modulate BK currents (pharmacological-, Ca^{2+} - sensitivities, kinetics…) in others cell types (for review see Ghatta et al., 2005). Here we investigated on possible beta subunit modulation of the mature rat IHCs BK channels, using whole-cell patch clamp technique and immunological staining.
We found that IbTX incompletely blocks the BK currents, even when applied for long period time, suggesting beta subunit presence. Indeed, it is known that when beta subunit can confer resistance to IbTX, due to their large extracellular domain that occludes the toxin binding site onto the pore (Behrens et al., 2000). Another piece of evidence for beta subunits involvement in IHCs is the activating effect of tamoxifen, which requires beta subunits (Dick et al., 2001). Our immunostainings results confirm an alpha localization in the IHC extra synaptic neck region (Hafidi et al., 2005; Pyott et al., 2005). Beta-1, -2, -3 and -4 subunits have been found in the IHC region, but at this stage of the study, no precise localization could be assessed. Beta expression within the IHC region suggests possible co-localization and interaction with the pore-forming alpha subunit.

P17

Single channel pKir inwardly rectifying currents from pigeon vestibular hair cells

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In a previous study the pigeon inward rectifier K channel pKir2.1 was cloned and its elementary properties investigated in a heterologous cell expression system (Correia et al., Physiol Genomics, 2004). We now have obtained single channel cell-attached recordings from pKir in semicircular canal type II native hair cells in an ampullary slice preparation from the adult pigeon. Ninety percent of the cells were from Zone III (center of the ampullary crista). The patch pipettes contained (mM): KCl 140, $MgCl_2$ 2, $CaCl_2$ 1, EGTA 11, HEPES 10 (pH 7.4 with KOH). The cell membrane voltage was zeroed by superfusing a bath solution with high-K^+ content (140 mM) onto the slice. In all experiments, the pipette contained ZD 7288 (66 or 33 microM), an inhibitor of Ih. Hyperpolarizing and depolarizing voltages were applied through the pipette. Inward current channel openings were observed for membrane voltages below –30 mV. Multi-level records were obtained, as reported for Kir in various tissues, including the Kir2.1 subfamily members. The records showed openings to at least 2 levels (large and small openings) and closings to at least two closed states and one sub-conductance level. The mean slope conductance was 30 pS for the large open state and 20 pS for the small open state (voltage range from –140 mV to –60 mV). The mean open time (to) and the mean open probability (Po) decreased with hyperpolarization from –40 mV to –140 mV: Po was 0.13 at –50 mV, and 0.03 at –120 mV; to was 42 ms at –50 mV and 3 ms at –100 mV. During pulse protocols between –100 and –140 mV, the mean current showed a decay, which was faster and stronger at more negative potentials. The decay constants of mean single channel currents were 46 ms at –100 mV, and 8 ms at –120 mV.
As far as the small conductance level is concerned, it might be due to Kir partial block by Mg^{2+} as found in mouse cardiac myocytes (Picones et al., Biophys J 2001).

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Neurotransmission

P18-P29

P18

Temperature enhances exocytosis efficiency at the inner hair cell ribbon synapse

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Hearing relies on fast and sustained neurotransmitter release from inner hair cells (IHCs) onto the afferent auditory nerve fibers. Little is known about the biophysical properties of the hair cell ribbon synapse at physiological temperature, because most in vitro experiments have been performed at room temperature so far. Here we performed perforated patch-clamp recordings of voltage-gated L-type Ca^{2+} influx and exocytic membrane capacitance changes at room (25℃) and physiological (35-37℃) temperatures. We show that temperature increases the L-type Ca^{2+} current of IHCs (Q10 = 1.3). We obtained more exocytosis per Ca^{2+} influx at physiological temperature. The amplitude of fast exocytosis (response to 20 ms depolarization, Q10, fast = 2.1) and the rate of sustained exocytosis (exocytic rate between 20 and 100 ms of depolarization, Q10, sustained rate = 1.7) were elevated disproportional to the increase in Ca^{2+} influx. We suggest that the number of readily releasable vesicles available at the active zone and the rate of their re-supply are higher at physiological temperature. We conclude that the efficiency of exocytosis is higher at physiological temperature than at room temperature.

P19

Analysing the Ca^{2+} currents in inner and outer hair cells of mice lacking the beta3- or beta4 auxiliary Ca^{2+} channel beta subunit

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Voltage-dependent Ca^{2+} channels (Cav) consist of the pore-forming alpha1 subunit (SU) and auxiliary SUs beta, alpha2-delta and gamma. The intracellular beta SUs are responsible for membrane trafficking of alpha1 SUs and modulate current amplitude and kinetics. Ca^{2+} currents in cochlear inner (IHC) and outer (OHC) hair cells are L-type currents and almost exclusively flow through the alpha1 SU Cav1.3. They are therefore a suitable system to test if there is a preference of this particular alpha1 SU for a beta-SU and if the lack of one of the SUs can be compensated by another one. Previous data have shown the presence of the mRNA of all four beta SU in hair cell-specific cDNA with a preference of beta1 and beta3 in IHC and OHC cDNA.
Ba^{2+} currents (IBa) were measured in neonatal IHCs and OHCs (P1-P7) and in mature IHCs (P18-P20) in beta3-/- and beta4-/- mice and controls. IBa amplitudes, voltage-dependence of activation, activation time constants and inactivation after 300 ms depolarization were determined. In beta3-/- mice, there was no difference in these parameters neither in IHCs nor OHCs except a higher inactivation in beta3-/- OHCs (7.7±4.6%, n=6) compared to control (2.2±1.5%, n=5). In beta4-/- mice, no difference in these parameters could be detected neither in IHCs nor OHCs except a reduction of IBa in mature beta4-/- IHCs (213±78pA, n=13) compared to IBa in beta4+/+ IHCs (284±78pA, n=12).
These subtle differences did not affect hearing thresholds of 3 week old beta3-/- and beta4-/- mice.
In conclusion, Cav1.3 channels IHC and OHCs either do not contain a considerable degree of beta3/beta4 or the function of beta3/4 can be compensated by other beta SUs.

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P20

Opioid receptor modulation of the calcium current in cochlear outer hair cells

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There is evidence that opioid peptides are expressed in olivocochlear efferent neurons and that cochlear hair cells express opioid receptors. To characterize the cellular mechanism mediating opioid actions in the cochlea. The calcium current and their modulation by opioid peptides were investigated in acutely isolated rat outer hair cells (OHCs) using the whole-cell patch-clamp technique. The current-voltage (I-V) relationship of the Ca^{2+} current activated near -60 mV, and the current reached its maximum at -10 mV. BayK 8644 (5 µM) caused a 3.5-fold increase in peak Ca^{2+} currents. These results corroborated that the Ca^{2+} channels in OHCs have L-type characteristics. The kappa opioid receptor agonist U-50488 (1 µM at 1 nM) (N = 27) decreased Ca^{2+} currents in a dose dependent form with an IC50 = 2 nM and a Hill number of 0.47. The U-50488 effect was partially reversible. The kappa opioid receptor antagonist norbinaltorphimine (1 µM) antagonized the action of 1µM U-50488. In contrast, both the mu- and delta opioid receptor agonists DAMGO (1 µM, N = 7) and DPDPE (1 µM, N = 6) had no significant effects on the Ca^{2+} currents. These results indicate that kappa opioid receptors activation modulates Ca^{2+} current in the OHC thus influencing intracellular Ca^{2+} level, neurotransmitter release and eventually the cell excitability via the Ca^{2+} activated K^{+} current. Opioid modulation of auditory function may constitute a significant mechanism in controlling auditory sensitivity and it may have a significant role in pathological process such as hyperacusis and tinnitus.

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P21

Fast and sustained exocytosis of inner hair cells co-vary with the synapse number along the tonotopic axis of the mouse cochlea

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The sensitivity of sound perception in the mammalian ear is highly dependent on frequency and – consequently – the corresponding location on the cochlea. Here, we investigated whether the morphological and physiological properties of the afferent hair cell synapses could contribute to this phenomenon. We found that the number of synaptic contacts per inner hair cell had a maximum in the cochlear region that transmits sounds with highest sensitivity (10-16 kHz). The number of synaptic contacts was determined by immunostaining for ribeye, a major component of the synaptic ribbon, and by identifying colocalized immunoreactivity for AMPA-receptor subunits GluR2 and 3 as postsynaptic markers.
We then performed perforated patch-clamp recordings from inner hair cells at different positions along the apical turn of the cochlea. Probing exocytosis by measuring cell capacitance increase after a brief depolarization, we found that hair cells located 300 µm from the apex – thus responsive for very low frequencies – released 44% less transmitter than cells located at ~1400 µm from the apex. Concomitantly, we observed 31% less afferent synapses in the most apical hair cells. To confirm, that the increase in synaptic transmitter release does not originate from an increase in the size of the presynaptic ribbons, we employed 4Pi high-resolution optical microscopy to measure the ribbon size distribution. Ribbons at different positions were indistinguishable from each other with distributions of identical means and half-widths. Interestingly, size, charge and kinetics of the calcium current did not vary with the tonotopic position of the hair cells. A possible explanation would be that a significant number of calcium channels is located extrasynaptically.

P22

Defective synaptic maturation in the inner hair cells of athyroid Pax8$^{-/-}$ mice

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Inner hair cells (IHCs) are the genuine sensory cells of the cochlea encoding sound information into neuronal signals at their afferent synapses. These synapses undergo major structural changes during ontogenesis, resulting in very different functional properties. Before the onset of hearing (P12 in mice) IHCs fire Ca^{2+} action potentials (APs) driving exocytosis at multi-ribbon active zones. Immature IHCs express a high density of CaV1.3 Ca^{2+} channels toward the end of the first postnatal week and mainly KV-type K^+ currents. The Ca^{2+} current density subsequently declines to lower levels in IHCs of hearing mice, which display single ribbon active zones and express BK K^+ channels that ensure graded potentials. Thyroid hormone (TH) could mediate this remodeling.
In this study we performed perforated patch-clamp recordings of the Ca^{2+} currents and exocytic responses in IHCs of P15 athyroid Pax8-/- mice, which serve as an excellent model of congenital hypothyroid deafness. We found several signs of immaturity in these mice. Mutant IHCs showed increased Ca^{2+} currents and exocytosis, suggestive of an immature stimulus-secretion coupling. In whole-cell mode experiments, mutant IHCs lacked fast BK-type K^+ currents and showed only slow KV-type K^+ currents. Current-clamp recordings revealed the persistence of APs and IPSPs in mutant IHCs. Immunohistochemistry and quantitative RT-PCR demonstrated an immature molecular synaptic organization in mutant IHCs at P15. These results, although preliminary, implicate TH as an important signal involved in the maturation of IHCs and their ribbon synapses as one prerequisite for normal hearing.

P23

Role of synaptic ribbons in hair cell sound coding

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The synaptic ribbon is an electron-dense structure surrounded by synaptic vesicles found at hair cell afferent synapses. In a knockout mouse with targeted deletion of Bassoon, the gene for a synaptic scaffolding protein, ribbons are no longer tethered to the presynaptic membrane. This loss of active-zone anchored ribbons coincided with a strong reduction of synchronous hair cell exocytosis in vitro and a degraded compound action potential, suggesting that the synaptic ribbon stabilizes a large readily releasable pool (RRP) of vesicles, thereby increasing the likelihood of multi-vesicular release and reducing the jitter of postsynaptic spiking.
Here we test this hypothesis of reduced temporal precision at the level of single auditory nerve (AN) fibers of Bassoon mutants in vivo. AN tuning appeared normal in mutants except at high frequencies. AN discharge retained the normal stochastic pattern seen in interval histograms of spontaneous activity, but spontaneous rates were reduced. Sound-evoked discharge was assessed by responses to tone bursts at 30 dB re threshold at CF. Although mutant fibers showed sustained responses, even to 5 sec stimuli, both steady-state and onset rates were reduced. In line with our hypothesis, distributions of first spike latencies showed increased variance in the mutants, while the mode was not different from wildtype. Presynaptic recordings of inner hair cell (IHC) potassium currents and membrane potentials indicated that the membrane time constant was unchanged. Immunohistochemistry showed that Ca^{2+} channels remain clustered at the synapse.
In conclusion, our data indicate that postsynaptic detection of coincident release of multiple vesicles contributes to the synapse's superior temporal acuity. As a result, the reduced RRP of ribbon deficient synapses lowers the rate of AN discharge and causes a degradation in the temporal precision of sound coding.

P24

Glutamate increases cochlear dopaminergic neurotransmission through NMDA receptors and NO release

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Glutamate (Glu), transmitter of the inner hair cells, can restrain its own excitatory effect on cochlear afferent nerves by activating a negative short-loop feed back through the brainstem, which results in the release of dopamine (DA) from lateral olivocochlear (LOC) terminals. DA is suggested to have neuroprotective effect against the excessive amount of Glu. Glu also activates the NMDA receptor-linked nitric oxide synthase (NOS). The produced nitric oxide (NO) is highly diffusible and known to influence DAergic neurotransmission in the central nervous system by the inhibition of DA reuptake.

The present study was designed to explore the local DA releasing effect of Glu within the cochlea and to determine the possible role of NO in this process.

Using an in vitro microvolume superfusion method, we measured the release of [3H]DA from isolated mouse cochlea.

We found that NMDA (10 and 100 µM) significantly increased the electrical-field stimulation-evoked release of DA from the mouse cochlea preparation. The NO donor sodium nitroprusside (300 µM) also enhanced the release of DA, while inhibition of the endogenous NO generetion by L-NAME was without any significant effect by itself. However, inhibition of NOS (L-NAME, 100 µM) significantly decreased, although not blocked, the effect of NMDA, suggesting that NO play a role in NMDA action. Enhancing effect of the selective DA uptake blocker nomifensine confirmed that inhibition of the reuptake has a significant effect on evoked DA release in the cochlea.

We obtained functional evidence that Glu can also increase cochlear release of DA by a local action which probably contributes to the regulation of Glu effect through the short loop feed back. The mechanism of this local effect involves the inhibition of DA reuptake by NMDA induced NO generation. Direct activation of NMDA receptors on the LOC efferent terminals may also play a role.

P25

Maturational changes at the inner hair cell ribbon synapse

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Inner hair cell (IHC) synapses play a key role in the auditory physiology as it ensures the transmission of sound stimuli to the first auditory neurons. Glutamate is the neurotransmitter responsible for its fast synaptic transmission which essentially involves AMPA receptors. The glutamate release is dependent on L-type Ca^{2+} channels with Cav1.3 subunit and occurs at synapses equipped with a dense ribbon thought to mediate the continuous and rapid recruitment of its attached vesicles to the release sites. Despite the importance of the IHC synapse, the cellular and molecular machineries underlying its function are still largely unknown despite their elucidation is of prime importance to gain insight into the occurrence of tinnitus and most forms of deafness.
We have studied here using immunocytochemistry the expression of pre- and postsynaptic proteins (SNARE, synaptic vesicle proteins...) during the postnatal maturation of the rodent cochlea and found that their expression was delayed by several days with respect to the establishment of the IHC synaptic contacts with primary auditory neurons dendrites (occuring around E18-E19). For example, synaptogyrin and cysteine-string protein were first expressed between postnatal days 10 and 12, when the first, immature, cochlear potentials can be recorded. During this maturational period, we also found that the composition and pharmacological properties of the postsynaptic AMPA receptors changed. GluR2 replaced GluR1 at postnatal day 10, switching the receptor composition from GluR1/3/4 to GluR2/3/4 and its pharmacology to calcium impermeability.
Finally, we have checked the expression of these proteins in the cochlea of the deaf Cav1.3 knock out mice and found that all these proteins were expressed in adult IHC synapses suggesting that their expression was not dependent of the first sound stimuli transduced by IHCs.

P26

Effects of human defensin NP-1 on synaptic transmission in the frog vestibular organs

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Defensins are a group of low-molecular-weight cationic antibacterial peptides isolated from animals and human neutrophils. These peptides have broad spectrum of antibacterial activity and also demonstrate immunoregulatory properties. A role of defensins in the transduction mechanisms in sense organs remains obscure. The present study attempts to characterize synaptic glutamate receptors of sensory epithelia by examining the effects of defensins on synaptic transmission and chemically induced afferent activity in vestibular organs of the frog.

Experiments were performed on isolated preparation of semicircular organs in the frog Rana temporaria during continuous external perfusion with normal and test solutions. Multiunit recording of action potentials from afferent nerve fibres was performed with a suction glass electrode. The test chemical was human neutrophil defensin NP-1.

Application of defensin at concentrations between 0.0001-10 nM depressed both the resting discharge frequency and L-GLU-induced facilitation. Addition of defensin to the L-GLU-containing solution inhibited the initial frequency increase under L-GLU and emphasized the frequency decrease that followed the excitatory phase of the response. For most units, the threshold concentration was 0.0001 nM. Firing evoked by kainate, AMPA, NMDA or ACPD could be depressed by administration of 1 nM defensin, demonstrating that both ionotropic and metabotropic subtypes of L-GLU receptors can be modulated by defensins in an inhibitory way. The specific opioid receptor antagonist naloxone antagonized the inhibitory response evoked by defensin, suggesting that defensins may be involved in the interaction with opioid receptors on hair cells.

The results obtained support the evidence for the recruitment of defensins in communication between the immune and nervous systems, and on the potential of sensory receptors to participate in the inflammatory response.

P27

Immunocytochemical and pharmacological studies of the metabotropic glutamate receptors in the frog semicircular canals

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Our study involving immunocytochemistry and multiunit recording of afferent activity of the whole vestibular nerve was focused on the postsynaptic role of the metabotropic glutamate receptors (mGluR) in the afferent neurotransmission of the frog semicircular canals. Both immunoperoxidase and immunofluorescent labeling revealed intense mGluR1a and mGluR2/3 immunoreactivity in all vestibular ganglion neurons. These results can be attributed to a postsynaptic localization of mGluR at the afferent terminals.

The experiments showed that mGluR agonist ACPD (0.01-1 mM) enhanced the resting discharge frequency and broad spectrum mGluR antagonist MCPG (0.05-1 mM) led to a concentration-dependent decrease of both resting activity and ACPD-induced responses. Responses elicited by ACPD were susceptible to the level of activation of the postsynaptic membrane. Indeed, ACPD demonstrated the facilitatory effect upon resting frequency induced by L-Glutamate in high-Mg2+-solution suggesting that ACPD acts on postsynaptic level. The firing induced by L-Glutamate in high-Mg2+-solution corresponds well to in vivo conditions of continuous transmitter release by vestibular hair cells and a stationary depolarization of the postsynaptic membrane.

To determine whether activation of mGluR modulates excitatory glutamatergic transmission, the ionotropic L-glutamate receptor (iGluR) agonists AMPA and NMDA were tested during a continuous application of ACPD. In such conditions, ACPD significantly potentiated the responses to AMPA and NMDA implicating mGluR in the excitatory control over both types of iGluR. These findings do not exclude the possibility of additional presynaptic mGluR effects (Guth et al. 1998; Hendricson and Guth, 2002a, b).

Thus, this study suggests that mGluR are not involved directly in rapid excitatory neurotransmission, which is mediated by iGluR. However, activation of mGluR potentiates AMPA and NMDA responses through a postsynaptic interaction.

P28

Evidence of functional purinergic receptors (P2X) in rat vestibular ganglion neurons

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Here we demonstrate, for the first time, the expression of purinergic receptors (P2X) on rat vestibular ganglion neurons (VGNs) using the whole-cell patch-clamp recording and RT-PCR.
Vestibular ganglia were prepared from newborn rats (P5-P10). The ganglia were incubated in activated papain (20U/ml) and dissociated by gentle trituration. Records were obtained from larger round-shaped neurons during 4-10 hours after dissection. Agonists and antagonists were delivered via linear array of three square tubes (tip diameter 100-150 µm), positioned within 200 µm of cells, and solution flow was controlled by gravity.
Under the whole-cell voltage-clamp recording condition, application of adenosine 5'-triphosphate (ATP; 100 µM) evoked desensitizing inward currents in ca. 80% of VGNs at a holding potential of -60 mV. Irrigating VGNs with the bath solution for 5 min recovered the initial response to ATP. Suramin (100 µM), a P2-purinergic antagonist, reversibly inhibited ATP-evoked inward currents. Alpha,beta-methylene ATP (100 µM), a P2X-specific agonist, evoked inward currents but had a lower potency than ATP. The time constant (τ) of the decay phase of ATP-evoked currents was 2-3 s, a value between those of rapidly desensitizing subgroups (P2X1 and P2X3) and slowly desensitizing subgroups (P2X2, P2X4, etc.), suggesting heterogeneous expression of P2X receptors in rat VGNs. Expressions of P2X1, X3, X4 receptor subunit were confirmed by the RT-PCR. The physiological implication of P2X receptors includes modulation of excitability at the synapse between hair cells and dendrites and/or trophic support (or also neuromodulation) from supporting cells surrounding the VGNs.

P29

New insights in the vestibular calyceal neurotransmission

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Glutamate is thought to be the main neurotransmitter at the synapse between the type I vestibular hair cell and its cognate calyx afferent. The present study was designed to identify the type of glutamate receptors involved in neurotransmission at this unusual synapse. Immunocytochemistry showed that AMPA GluR2, NMDA NR1 and NR2A/B subunits of the glutamate receptors were confined to the synaptic contact. We then examined the electrical activity at calyx terminals using direct electrophysiological recordings from intact dendritic terminals in explanted turtle posterior crista. We found that sodium-based action potentials support a background discharge that could be modulated by the mechanical stimulation of the hair bundle of the sensory cells. These activities were prevented by blocking both the mechano-electrical transduction channels and L-type voltage-gated Ca^{2+} channels involved in synaptic transmission. Although pharmacological analysis revealed that NMDA receptors could operate, our results show that AMPA receptors are mainly involved in synaptic neurotransmission. We conclude that although both AMPA and NMDA glutamate receptor subunits are present at the calyx synapse, only AMPA receptors are involved in the synaptic transmission between the type I vestibular hair cell and the afferent calyx.

Mechanisms of Development

P30-P39

P30

Notch signaling inhibitors increase hair cells in embryonic organ culture

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Introduction: In mammals, there has been no therapeutics for hair cell damage in inner ears. To achieve regeneration of hair cells, it is first important to elucidate molecular mechanisms underlying hair cell development. Notch signaling controls cell differentiation in a variety of mammalian tissues. During inner ear development, Notch signaling has been implicated to regulate cell fate choice between hair cells and supporting cells, by studying mice deficient in Notch receptors and ligands. However, precise mechanisms how Notch signaling control such cell differentiation processes in inner ears are largely unknown. Here, we develop a novel in vitro culture of mouse fetal cochleas, and addressed regulation of inner ear development by Notch signaling using inhibitors for two proteases, g-secretase and TACE, both of which are independently required for Notch activation.
Method: Mouse embryonic cochleas were prepared, and then cultured in vitro in the presence or absence of g-secretase or TACE inhibitor to block the Notch signaling. After culture, immunohistochemistry and quantitative RT-PCR were performed.
Result: Notch signaling inhibitors increase cochlear hair cells from E14.5. Notch signaling controls binary cell fate choice between hair cells and supporting cells. This control is mediated by induction of hes1 or hes5, and suppression of atoh1. In addition, Notch controls proliferation of putative progenitors.

P31

mRNA expression of growth factors in the organ of Corti, modiolus and stria vascularis of newborn rats

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The results of several studies indicate that growth factors are important regulators of the inner ear development and are involved in cell death and survival. In order to analyze the basal expression of growth factors by the main cochlear structures and its intrinsic potential to response to cochlear injury and hypoxia, we studied freshly prepared cochlear structures and their corresponding organotypic cultures.
Organ of Corti (OC), modiolus (MOD) and stria vascularis (SV) were prepared from neonatal rats (3-5 days old) and analyzed immediately after preparation and 24 h after organotypic cultivation. The Affymetrix Rat Neurobiology U34 microarray chip (MA, total of 1322 gene transcripts; two independent samples) was used to screen the mRNA expression levels of 70 gene transcripts coding for growth factors from different gene families.
Genes with an expression level above the upper quartile of the normalized signals of all growth factors on the chip were analyzed. The members of the insulin-like growth factor system Igf1, Igf2 and Igfbp2 were highly expressed in all regions. In the OC, cultivation decreased the expression of growth-associated protein 43/Gap43, neurotrophic tyrosine kinase receptor 2/Ntrk2 and fibroblast growth factor receptor 4/Fgfr4. In the MOD, cultivation decreased the expression of Igfbp2 and transforming growth factor beta 3/Tgfb3. In the SV, cultivation decreased the expression of Fgfr4. Cultivation increased the expression of Igfbp5 and intercellular adhesion molecule Icam1 in the OC, Igfbp3, Igfbp5 and Icam1 in the MOD and the SV. Hypoxia-exposed cultures showed similar changes.
The data indicate that the IGF system has a dominating role to play in the normal development of the inner ear. The cochlea responds to the preparatory injury and cultivation by down- and up-regulation of several growth factors indicating the effective adaptation to in vitro conditions and to hypoxia.

P32

Developmental changes of cross-link arrangement and BAPTA sensitivity in rat cochlear hair bundles

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In the inner ear incoming mechanical stimulations were transformed by hair cells into electrical signals directed to the brain. This mechanoelectrical transduction (MET) is based on a molecular complex responding to sound by opening a mechanosensitive ion channel. A filamentous extracellular structure – called tip link – is thought to directly pull at the channel's pore in response to sound while cross-links hold the hair bundle in shape. Since an intact morphology of hair bundles is essential for hearing we investigated the developmental changes in cross-link arrangement and morphology in the mammalian cochlea using high-resolution scanning electron microscopy. In the rat the organ of hearing maturates within several weeks after birth to adult-like appearance with the onset of hearing at about twelve days after birth (P12). A huge variation in cross-link arrangement during this postnatal maturation was observed in inner and outer hair cells. In the early stage of postnatal development a dense network of cross-links between stereocilia of cochlear hair bundles was seen. However a clear differentiation between potential tip links and side-to-side links was only possible for hair cells with stereocilia graded in height. The question whether cross-links are already differentiated into different classes of links before the onset of hearing was addressed by a pharmacological study. Application of 5 mM BAPTA, a known tip link disrupting calcium-chelator, to the organ of Corti showed a big developmental difference in the sensitivity of cross-links. The biggest effect of cross-link disruption was found in the very early postnatal stage at day one after birth, while cross-links of adult hair bundles showed a more selective disruption of links. Our results do not only suggest a reduction in the number of cross-links and an increase in morphological organization during development but also a differentiation into different classes of cross-links with variable BAPTA-sensitivity.

P33

Age-related apoptotic change in the cochlear lateral wall of mice

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Apoptosis is thought to be involved in the aged inner ear disturbance. Lateral wall of cochlea has an important role to maintain the homeostasis of the inner ear. We examined apoptotic changes in the lateral wall of mice cochlea using electrophysiological and immunohistochemical techniques.
Twenty-four mice (129X1/SVJ strain) were used in this study. The auditory brain stem response was recorded at the age of 1, 4, 8 and 12 months. On each period, 6 mice were served for the immunohistochemical study. Fragments of DNA were detected with a specific antibody to single-stranded DNA.
The threshold of the auditory brainstem response tended to worsen at the age of 4 months. This threshold shift became significant at the age of 8 and 12 months. We detected fragments of DNA in the lateral wall, mainly in the spiral ligament, of the cochlea at the age of 4, 8 and 12 months. The number of cells that stained positive for single-stranded DNA, increased according to the aging.
Our findings indicate that apoptosis participates in the aged cochlea, especially in the spiral ligament and that this phenomenon contributes to the pathogenesis of presbycusis.

P34

Developmental BK channel distribution in relation to the appearance of square arrays in the lateral membranes of mice inner hair cells

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Large-conductance, calcium-activated potassium (BK) channels, have been localised close to the cuticular plate in the lateral membrane of mature mammalian inner hair cells (IHC). In amphibians, the gradation in distribution of BK channels in hair cells along the basilar papilla has been suggested a role in determining tonotopicity. Their role in IHC is less well understood but they may contribute to the cessation of action potential induction in immature hair cells. For this to occur, a dense insertion near the region of potassium ion entry would be propitious for modulating the degree of depolarization. Freeze-fracture electron microscopy has previously shown that the lateral plasma membrane of IHC is uniquely characterised by the presence of closely packed particles in square arrays that are confined to the supranuclear region of the cell just below the tight junctional complex. The fact that the particles span the plasma membrane has led to speculation that they may represent transmembrane channels. Functional BK channels are thought to appear just before the onset of hearing in mice – at around P12, and immunocytochemical studies have reported the appearance of BK channels at the apical ends of IHC at around the same time. Thus using mice of different ages, ranging from P0 to mature, we compared the appearance and distribution of immunolabelling for BK channels using a polyclonal antibody specific to slo channels (the Bk alpha subunits) with that of square arrays of particles on the fracture faces of IHC lateral plasma membranes. In mature animals immunohistochemistry revealed distinct patches of labelling near the cuticular plate that coincided in size and distribution with the square arrays exposed by freeze-fracture. Freeze-fracture showed the square arrays first became apparent on IHC membranes at around the time of onset of hearing. It seems likely that the BK channels in IHC are organised in distinctive plaques.

P35

Estrogen receptors in the inner ear during different stages of pregnancy

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Background: Is the female sex steroid estrogen the key to preserved hearing in the aging human? Hearing loss is more profound in elderly males than females. There are sex differences in the ABR, where women have shorter latencies. Women at menopause with hormonal replacement therapy have a better hearing than non-substituted and women with Turner syndrome (45,X), who are biologically estrogen deficient, show longer ABR latencies and an early presbyacusis. In animal experiments, the female midshipman fish without estrogen, turn a deaf ear to the songs of the males but when boosted they can hear the males' call. If estrogen receptor beta is knocked out, a severe progressive hearing loss is present in mouse. There are two estrogen receptors, alfa and beta both of which are present in the inner ear of mouse, rat and humans. Knowing how sex steroids can alter the hearing abilities might give important clues as to how estrogen can preserve hearing in humans.
Aim: Thirty-four rats, in different time periods of pregnancy and 8 fetuses have been investigated in order to study the effect of estrogen receptors and estrogen on the inner ear.
Methods: Rats were sacrificed and estrogen levels were sampled and inner ears studied immunohistochemically for estrogen receptors that were quantified.
Results: Estrogen receptors are present in the inner ear of the rat and differ during pregnancy and period. During early pregnancy (day 8), ER alfa is found in type I spiral ganglion cells, while ER beta in type II cells. During the late pregnancy (day 18) both ER alfa and beta were down regulated. No estrogen receptors could be found in the inner ear of the fetus.
- Before puberty both ER alfa and beta are present in the inner ear, but when the young adult rats (8 weeks) the ER? alfa and beta are down regulated.
- During pregnancy and development the estrogen levels vary which can be mirrored in the receptor regulation in the inner ear.

P36

Role of Sox10 in the development of the inner ear

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SOX10 is a member of the high-mobility group-domain SOX family of transcription factors and is implicated in the control of a variety of developmental processes. Disruption of neural crest development in the Dominant megacolon (Dom) mice is associated with a Sox10 mutation. Mutations in human Sox10 gene have also been linked with the occurrence of neurocristopathies in the Waardenburg-Shah syndrome type IV (WS-IV), for which the Sox10 (Dom) mice serve as a murine model. The neural crest disorders in the Sox10 (Dom) mice and WS-IV patients consist of hypopigmentation, cochlear neurosensory deafness, and enteric aganglionosis. Consistent with these observations, a critical role for Sox10 in the proper differentiation of neural crest-derived melanocytes and glia has been demonstrated. However, the role of Sox10 in the development of the inner ear remains unknown.
In this work we use the Sox10lacz mice where the entire reading frame of Sox10 is deleted and replaced by LacZ sequence allowing the visualization of its expression by β-galactosidase staining. Our experiments show that Sox10 is expressed in the spiral ganglion Schwann cells and in the melanocytes of the stria vascularis, both of which derived from the neural crest stem cell. But more surprisingly, we show that Sox10 is also expressed in the entire otic vesicule which comes from the otic placode, an embryonic ectoderm derivative. These results show that the transcriptional factor Sox10 is widely expressed in the embryonic inner ear suggesting that it plays a critical role in its development.

P37

Distribution of calbindin in the developing inner ear of the mouse

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Previously, the distribution of calbindin (CB), a 28 kDa Ca^{2+} binding protein, was reported in the inner ear of adult animals and in some cases also during prenatal development, in several mammalian species such as rat, dog, musk shrew, gerbil and guinea pig. In this study, the distribution of CB was investigated immunocytochemically during embryonic and early postnatal development in the mouse (strain C3H). Calbindin immunoreactivity first appeared on embryonic day 12 (E12) in the vestibulo-cochlear (VIIIth) ganglion complex and in the differentiating otocyst. During mid-gestation (E14), CB expression persisted in the VIIIth ganglion but was not detected in the cochlear duct. At E17 CB positivity appeared in the differentiating sensory epithelium of the inner ear. At E19 the CB staining became stronger in the cochlear duct and CB labeling appeared in developing hair cells and the stria vascularis. More cochlear neurons in the VIIIth ganglion were CB-positive in comparison with the vestibular neurons during the late embryonic period. Postnatal (P4) CB expression was detected in the cochlea; the inner and outer hair cells, the stria vascularis and spiral ganglion cells were all strongly positive. Also, vestibular hair cells expressed CB postnatally. In conclusion, CB is involved in the development of both neuronal and non-neuronal structures of the inner ear in the mouse.

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P38

Survival and differentiation of mouse embryonic statoacoustic ganglion cells in co-culture with auditory brain stem slices

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Most types of congenital and acquired hearing loss are caused by loss of sensory hair cells in the inner ear, or by loss of afferent neurons. The replacement of spiral ganglion neurons would be a fundamental step in any attempt to restore hearing loss. We have previously transplanted embryonic neuronal tissue and stem cells into inner ear to initiate a neuronal repair paradigm. Here, we have studied the survival and differentiation of mouse embryonic statoacoustic ganglion (SAG) cells into the rat postnatal brainstem slice culture. Our earlier studies have shown that in adherent monoculture, SAGs explants are able to differentiate into neurons and glial cells. In this study we have co-cultured SAG cells (GFP mice at E13) with auditory brainstem slices from Sprague-Dawley postnatal rats (P12-14). Using a tissue chopper, 300-µm-thick slices encompassing the vestibulocochlear nerve and cochlear nucleus were prepared and propagated using membrane interface methods as described by Stoppini (1991). In some experiments, cochlear nuclei were labeled by DiI. In vitro transplantation of SAG cells was conducted at culture day 5±2; 1-2 SAGs were deposited next to the vestibulocochlear nerve and cochlear nucleus. After two weeks, co-cultures were fixed and immunostained with antibodies raised against neural progenitors, neuronal, glial and synaptic vesicle markers. The results show that at two weeks of co-culture with auditory brain slices, SAG cells survived, proliferated, and differentiated into neurons and glial cells. Moreover, SAG cells migrated toward the cochlear nucleus, pre-labeled with DiI, and showed immunoreactivity for a synaptic vesicle marker, indicating the possible formation of functional synaptic connections between host and donor neurons. These results demonstrate that organotypic slice co-cultures of the auditory brainstem with SAG cells might be a useful model for studying regeneration of spiral ganglion neurons.

P39

Temporal and spatial regulation of α6 integrin expression during the development of the cochlear-vestibular ganglion

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The neurones of the cochlea-vestibular ganglion (CVG) that innervate the sensory hair cells of the inner ear are derived from the otic epithelium early in development. Following neuronal determination, neuroblasts detach from neighbouring cells and migrate into the anterior-ventral mesenchyme where they coalesce to form the ganglion complex prior to sending processes back into the epithelium. Cell migration and neuronal process formation involves changes in cellular interactions with other cells and proteins in the extracellular matrix that are orchestrated by cell surface-expressed adhesion molecules, including the integrins. Using immunohistochemistry and RT-PCR in murine tissue sections, otocyst and ganglion explants I have studied the expression pattern of the alpha 6 integrin subunit during the early development of the CVG. At embryonic day (E) 10.5 α6 integrin is expressed in the otic epithelium but not in migrating neuroblasts. Importantly, loss of alpha 6 was associated with epithelial exit rather than neural determination revealing differentiation cues acutely associated with cellular environment. Markers of glial and neuronal phenotype showed that alpha 6 expressing cells present in the CVG at this stage were glia of neural crest origin. By E12.5 α6 expression in the ganglion increased alongside the elaboration of neuronal processes. Immunohistochemistry in otocyst cultures in the absence of glia revealed that neuronal processes remained alpha 6-negative at this developmental stage and confirmed that alpha 6 was expressed by closely apposed glia. The spatio-temporal modulation of α6 expression suggests changing roles for this integrin during the early development of inner ear innervation.

Ototoxicity and Physiopathology

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Cochlear effects induced by cisplatin and oxaliplatin – a dose escalation study

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Introduction: Cisplatin is a potent anticancer drug but hearing loss is a dose limiting side effect. Oxaliplatin is a third generation platinum anticancer drug used in the treatment of colorectal carcinoma where cisplatin is ineffective. There is no earlier experimental or clinical report on oxaliplatin-induced injury to the inner ear. The aim of the study was to determine the dose-dependent toxicities of cisplatin and oxaliplatin. Materials and methods: 25 guinea pigs were divided into 5 groups. Cisplatin was tested at 5, 8 and 12.5 mg/kg and oxaliplatin was given at 2 equimolar doses 10.6, 16.6 mg/kg. Body weight was determined before and 96 hours after i.v injection. Evaluation of ototoxicity was based on ABR threshold shift and hair cell counts. Blood samples for creatinine analysis were taken before and 96 hours after injection. All animals were decapitated after 96 hours. One cochlea from each animal was prepared for cochleogram and the other for analysis of total platinum in the organ of Corti and stria vascularis. Results: After 96 hours there was no weight reduction of the animals given oxaliplatin whereas animals given cisplatin 12.5 mg/kg lost 11% of body weight. There was a slight increase of plasma creatinine level in animals given oxaliplatin 16.6 mg/kg. Increases in plasma creatinine were significant in all three groups given cisplatin. Oxaliplatin did not affect the ABR threshold or hair cell counts but cisplatin showed a significant effect in a dose-dependent manner. The total platinum concentration in the organ of Corti and stria vascularis of animals given the highest equimolar doses of cisplatin and oxaliplatin was 0.650 mg/kg and 0.02-0.2 mg/kg respectively. Discussion: The toxicities of cisplatin are related to the level of the single dose whereas equimolar doses of oxaliplatin show no ototoxic side effects. The lack of an ototoxic effect of oxaliplatin might be related to a low permeability for oxaliplatin through the blood-labyrinth barrier.

P41

Microarray data of the newborn rat cochlea and their changes during cultivation and hypoxia

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Introduction: The adaptation of cells and tissues to in vitro conditions and to hypoxia is linked to their gene expression patterns. Moreover, a cell culture model of the newborn rat cochlea is associated with cell survival and growth. Gene expression data of the organ of Corti (OC), the stria vascularis (SV) and the modiolus (MOD) as measured under cultivation and hypoxia have been lacking in the literature.
Methods: Microarray data (RN U34 chip, Affymetrix, total of 1322 transcripts) of newborn rats' OC, SV and MOD (2 pools of 6 cochleas each) - freshly prepared, cultivated for 24 h or exposed to hypoxia for 5 h (within the cultivation period of 24 h) - were statistically analyzed. The hypoxia-induced cochlear damage was evaluated by counting the hair cells (phalloidin staining).
Results: The significance of the microarray data was derived from comparing several duplicate samples. Altogether, the normalized signals of 263-316 genes were found to be present in the different regions ($p < 0.04$). During cultivation, 23-35 genes showed a 4-fold up-regulation and 7-20 genes showed a 0.25-fold down-regulation in the 3 cochlear parts. Similar changes in the OC and the SV and more pronounced ones in the MOD occurred. Hypoxia, which was associated with an obvious hair cell loss, induced only 1.6-fold and 0.5-fold changes in the expression of a small number of genes, especially in the SV and the MOD, as compared with cultivation.
Discussion: The RN U34 chip provides information on the neurobiological status of the cochlea. In the freshly prepared cochlea of postnatal days 3-5, the characteristics of the OC and the SV are already pronounced. The MOD gene expression pattern indicates immaturity. Obviously, the cultivation is associated with strong changes in the mRNA gene expression resulting in small additional changes during mild hypoxia.

P42

Expression of hypoxia-dependent genes in the cochlea of the newborn rat

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Background: Under hypoxia/ischemia, the transcription factor HIF-1 ('hypoxia-inducible factor-1') regulates the expression of a number of genes that are involved in glucose metabolism, cell proliferation, cell growth, apoptosis, oxidoreduction, angiogenesis and blood circulation. Because hypoxia may have an important role to play in the development of tinnitus and hearing loss, the expression of HIF-1-dependent genes in the cochlea were examined under normal and damaging conditions.
Methods: We used an in vitro model of the newborn rat cochlea and prepared the organ of Corti (OC), the stria vascularis (SV) and the modiolus (MOD). The mRNA expression was measured using the microarray technique (RN U34 chip, Affymetrix) under three conditions: 1) freshly prepared tissue, 2) after 24 h of cultivation and 3) 5 h of hypoxia during the 24-h cultivation period (n = 2 each). The findings were confirmed by reverse transcription PCR (RT-PCR; n = 6 each).
Results: The microarray data of OC, SV and MOD showed an increase of the mRNA levels of HIF-1alpha and of numerous HIF-1-dependent genes such as Gapdh/glyceraldehyde-3-phosphate dehydrogenase, Slc2a1/solute carrier family 2 (facilitated glucose transporter), member 1, Hmox1/heme oxygenase 1 and Nos2/inducible nitric oxide-synthase during the cultivation. Hypoxia (5 h) resulted in a further small increase in the mRNA expression of these genes only. The RT-PCR data closely confirmed the increases of Gapdh and Slc2a1 ($p < 0.001$) during cultivation and hypoxia. The Nos2 expression increased in the SV only. Hmox1 showed a low expression which was not changed by cultivation and hypoxia. Conclusion: The data revealed that the expression of HIF-1 dependent genes is strongly influenced by cultivation. Under these conditions, a mild hypoxia has only a minor effect on the expression of HIF-1-dependent genes. The increase in the Nos2 expression in the SV may indicate the role which NO has to play in the regulation of SV blood flow.

P43

Oxidative stress pathways in the potentiation of noise-induced hearing loss by acrylonitrile

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We hypothesize that disruption of antioxidant defenses is a key mechanism whereby chemical contaminants can potentiate noise-induced hearing loss (NIHL). This hypothesis was tested using acrylonitrile (ACN), a widely used industrial chemical whose metabolism is associated with significant potential for oxidative stress. ACN conjugates glutathione (GSH), depleting this important antioxidant. A second pathway involves oxidation by cytochrome CYP2E1 that leads to the formation of cyanide (CN). CN, in turn, can inhibit Cu/Zn superoxide dismutase. We have shown recently that ACN can potentiate NIHL and hair cell loss, even when noise levels approach permissible occupational exposure levels. However, the relative involvement of GSH depletion and CN production in this mechanism is still unknown. Determining the relative contribution of these two pathways by compromising the alternate branch pharmacologically may further delineate the role of specific antioxidants in the protection of the cochlea, thus enhancing the therapeutic possibilities. We investigated the effects of sodium thiosulfate (STS), a CN inhibitor, 4-methylpyrazole (4-MP), a drug that prevents ACN from being oxidized by CYP2E1, and L-N-acetylcysteine (L-NAC), a pro-GSH drug, in order to distinguish between GSH depletion and cyanide production as the mechanism responsible for the potentiation. Rats were exposed to noise (97dB, 8kHz centered octave band, 4hr/day, 5 days) and ACN (50mg/kg). Pre-treatment with STS (150mg/kg), 4-MP (100mg/kg) and L-NAC (4x400mg/kg) dramatically reduced blood CN levels, but only L-NAC induced a virtually complete protection of GSH levels in the liver and the cochlea. Concurrently, only L-NAC decreased the high-frequency auditory loss and hair cell damage resulting from ACN+noise, suggesting that GSH is involved in the protection of the cochlea against reactive oxygen species generated by moderate noise levels.

P44

siRNA-based elucidation of the impact of HIF-1α and HIF-2α on the expression of hypoxia-inducible genes in the organ of Corti of newborn rat

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Adaptation of cellular functions to hypoxic episodes is mainly regulated at the level of hypoxia-inducible transcription factors (HIF-1α, HIF-2α). At normoxia, HIF is down-regulated by the concert action of prolyl 4-hydroxylase PHD, E3-ubiquitin ligase pVHL and 26S proteasome. At hypoxia, this HIF degradation pathway is suppressed. The elevated HIF level induces the expression of adaptive target genes. The aim of our studies is to elucidate the regulatory impact of HIF-1α and HIF-2α on the expression of hypoxia-inducible genes in the organ of Corti of newborn rats.
The investigations are performed by using primary cell cultures prepared from the organ of Corti. The expression levels of HIF-1α and HIF-2α as well as of hypoxia-inducible genes at RNA level is determined by quantitative real time PCR. In the first step, siRNAs which are capable of specifically knocking-down HIF-1α and HIF-2α are evaluated, and in the second one, the expression kinetics of selected genes at hypoxia is analyzed. The transfection of the primary culture of the organ of Corti is optimized by using FITC-labeled siRNAs and different transfection reagents. From a panel of potential siRNAs, HIF-1α and HIF-2α specific siRNAs decreasing the mRNA level by up to 30% are identified. Expression kinetics of hypoxia-inducible genes is investigated within a time range of 24 hours. Hypoxia has no effect on the HIF-1α and HIF-2α mRNA expression. Among the genes investigated, the mRNA level of the facilitative glucose transporter 1 and that of glyceraldehyde-3-phosphate dehydrogenase reach their maximal levels at about 8 hours after the onset of hypoxia. HIF-1α and HIF-2α siRNAs are applied to elucidate the regulatory impact of HIF on hypoxia-inducible gene expression.

P45

Expression of the BK channel is severely delayed in IHCs of hypothyroid rats and shows a mosaic expression pattern

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Thyroid hormone (TH) is essential for the development of normal hearing. Lack of TH in a critical developmental period from E17 to P12 leads to morphological and functional deficits in the organ of Corti and the auditory pathway. We investigated the effects of TH deficiency on the expression of the fast activating K^+ current (IK,f) carried by the Ca^{2+}- and voltage activated K^+ (BK) channels in IHCs using patch-clamp recordings from IHCs of hypothyroid rats.

IHCs of control rats acquired a rapidly activating outward K^+ current and showed a steep increase in whole-cell K^+ current amplitude from P12 onwards which is consistent with the expression of BK channels around the onset of hearing. In contrast, K^+ currents of IHCs of hypothyroid rats did not increase substantially after P12 and did not show rapid current activation until P27. After P27, part of the IHCs from hypothyroid animals expressed IK,f while their neighbours did not. This mosaic pattern of expression could be confirmed by staining the BKα subunit with whole-mount immunocytochemistry.

Analysis of the kinetics of IK,f current activation revealed larger (approximately doubled) activation time constants (about twice as large) in hypothyroid IHCs compared to euthyroid controls. In control IHCs, 100 nM iberiotoxin blocked only part of IK,f. The activation time constants of these iberiotoxin-resistant fast K+ currents were similarly elevated as those of the fast K^+ currents of BK-expressing hypothyroid IHCs. This confirms heterogeneity of BK current properties in control IHCs (Marcotti et al., 2004, J Physiol 557, 613-633) and suggests incomplete acquisition of mature BK currents in hypothyroid IHCs. The different properties of BK currents could be caused by different splice variants of the BKα subunit or differential participation of BKβ subunits.

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P46

A mouse model for selective degeneration of the spiral ligament due to local application of 3-nitropropionic acid

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Recent studies in humans and animal models have indicated the importance of degeneration of the spiral ligament (SL) in the pathogenesis of sensorineural hearing loss. The aim of this study was set to establish a mouse model for selective degeneration of the spiral ligament. In this study, we examined auditory function and histology of mouse cochleae received local application of 3-nitropropionic acid (3-NP).
We injected solutions containing 3-NP at concentrations of 30, 100 and 300 mM into the posterior semicircular canal of C57BL6 mice. Auditory brain-stem responses (ABRs) were recorded at frequencies of 10, 20 and 40 kHz on days 1, 4, 7 and 14 after 3-NP application. The cochlear specimens were obtained on day 14 and provided for histological analyses.
Significant degeneration of the SL was observed in cochleae treated with 100 or 300 mM 3-NP. Degeneration was remarkable in the regions for type II and IV fibrocytes. Degeneration of the stria vascularis was not observed at any concentrations of 3-NP. Immunohistochemistry for Na, K-ATPase and connexin 26 demonstrated preservation of expression of these molecules in the cochlear lateral wall except for type II and IV fibrocytes regions of the SL. In cochleae treated with 300 mM 3-NP, significant loss of spiral ganglions and severe degeneration of the organ of Corti was identified. These findings indicate that selective degeneration of type II and IV fibrocytes regions of the SL occurs in cochleae treated with 100mM 3-NP. ABR measurements demonstrated permanent threshold shifts in 100 mM models, which were significantly lower than those in 300mM models. We then consider that selective degeneration of the SL may be a predominant cause for hearing loss in 100 mM models.
In conclusion, local application of 100 mM 3-NP causes selective degeneration of the SL and associated hearing loss, which may be a useful experimental model for investigating therapeutic strategies for SL degeneration.

P47

Differential gene expression of artemin, GDNF, BDNF and TGFbeta in deafened rats following electrical stimulation

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Degeneration of the auditory nerve secondary to the hair cell loss following drug- and noise-induced trauma is the effect of apoptosis induction in spiral ganglion cells (SGC). Unaffected cochlear inner hair cells (IHC) provide excitatory activation of the auditory nerve, and absence of both excitatory activation and trophic factors as like as neurotrophic factors has been implicated in the loss of auditory neurons. Reintroducing excitability to the auditory nerve following IHC loss using chronic cochlear electrical stimulation (ES) has been shown to restrict deafness-related loss of SGC. However, the SGC survival mechanisms induced by ES has not been completely clarified. Since neurotrophic factors, especially members of the GDNF-, NGF- and TGFbeta-families, play key roles for the protection of SGC in response to stress, the aim of the present study was to determine gene expression patterns of the GDNF family members, BDNF, TGFbeta1/2 and their receptors in the auditory nerve of deafened rats following chronic electrical stimulation. Adult rats were deafened by local inner ear injection of 10% neomycin, implanted with a platin-iridium-ball electrode and sacrificed following 21 d stimulation. The gene expression of artemin (ART), GDNF, persephin (PSPN), neurturin (NRTN), BDNF, TGFbeta1/2 and their receptors GFRalpha1-alpha3, Ret, trk B and TGFbeta receptors type I and II (TGFbetaR1/2) was determined by semiquantitative RT-PCR. In summary ART, GDNF, BDNF, TGFbeta1 and the receptor GFRalpha1 were significantly upregulated following ES. Only the main receptor of BDNF, trk B, was downregulated. Our data indicate key functions of ART, GDNF, BDNF and TGFbeta not only in stress response, but especially in supporting recovery of excitatory activity of the auditory nerve following ototoxic trauma.

P48

Cisplatin-induced gene expression in the rat cochlea

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Cisplatin (cis-diaminodichloroplatinum) is a widely used chemotherapeutic agent but unfortunately the use is limited by its ototoxic and nephrotoxic effects. In addition to the DNA-damaging effects, cisplatin induces oxidative stress to cells of the inner ear, leading to mitochondrial damage and activation of cell death pathways. The aim of the present study was to explore the molecular signaling pathways in the cochlea by studying gene regulation 96 hours after a cisplatin insult. Female Sprague Dawley rats were anesthetized and injected through the left jugular vein with cisplatin (16 mg/kg). Control animals received equivalent volumes of saline. Cisplatin treatment induced a mean threshold shift of 46 ± 4 dB (mean ± std) as recorded by auditory brainstem responses. Ninety six hours after the injection, animals were sacrificed and the soft tissue of the cochlea, including the organ of Corti, stria vascularis and spiral ganglion, was dissected out and RNA was isolated and used for gene expression analysis. RNA extracted from a single cochlea was used on each microarray in order to identify the biological variation and to be able to correlate the induced threshold shifts to the gene regulation in individual animals. RNA from three control- and three cisplatin-treated animals (a minimum of 100 ng total RNA in each sample) was hybridized to the GeneChip Rat 230A (Affymetrix) using Affymetrix small sample protocol. The statistical significance of differentially expressed genes was tested using a t-test, and the resulting list of genes sorted according to functional category in Gene Ontology.
The long-term aim is that this approach will lead to new therapeutic interventions for cisplatin-induced hearing loss. Future therapies could work by blocking cell death pathways or stimulating protective or regenerative pathways.

P49

Relationship between noise-induced hearing loss, enhanced amplitudes of cortical evoked responses and gap detection threshold changes

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Noise exposure produces disorders not only in the peripheral, but also in the central part of the auditory system. Whereas elevated hearing thresholds after acoustic trauma are connected to a great extent with damage to the inner ear, the enhanced amplitudes of cortical middle latency responses (MLR) observed after noise exposure in most animals indicate pathological changes in the higher levels of the auditory system. It has been shown previously that MLR amplitude enhancement in many cases is accompanied by a worsening of the gap detection threshold (GDT), which indicates the presence of tinnitus after acoustic trauma. The purpose of this work was to study the relationship between enhanced MLR amplitudes and worsened gap detection after acoustic trauma, specifically following repeated noise exposure in rats. The rats were exposed for one hour to broad-band noise of 118 dB SPL (first exposure) and 122 dB SPL (second exposure). The interval between the noise exposures was three weeks. Changes in hearing threshold and MLR amplitudes recorded in response to tone stimuli were compared with changes in GDT after noise exposure. The first noise exposure resulted in temporarily elevated hearing thresholds and GDT, in most cases accompanied by MLR amplitude enhancement of 150-350%. One day after noise exposure maximal hearing threshold shifts at high frequencies ranged between 25-45 dB and GDT values increased from 1.6 ms to 3-10 ms. Full recovery to normal pre-exposure values was observed during two weeks. The second exposure to more intense noise resulted in more pronounced changes in GDT (up to 40 ms) with a maximal hearing threshold shift of about 80 dB. However, the MLR amplitude enhancement was similar as that measured after the first noise exposure and did not increase further. The results demonstrate that the enhanced GDT and enhanced MLR after acoustic trauma develop independently.

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P50

Macrophage invasion in the amikacin treated rat cochlea

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Recent studies have proven the capacity of supporting cells to trans-differentiate into new hair cells in the mammalian cochlea (Izumikawa et al., Nat Med 11, 2005; White et al., Nature 44, 2006). This makes the supporting cells potential targets for therapeutic manipulation. However, damage to hair cells caused by noise or ototoxic exposure is generally followed by supporting cell disappearance. Understanding the reasons for supporting cells degeneration may help to promote the success of potential therapies.
Scavenger cells may have positive effects on tissue reparation through cell residues elimination and cytokine release, but they could have deleterious effects on remaining epithelial structures via autoimmune and inflammatory responses. In the noise or ototoxic exposed cochleas, they could exacerbate the initial damage.
The goal of this study was to investigate the organ of Corti of amikacin treated rats for the presence of macrophages. The density of macrophages was assessed from the end of the amikacin treatment until the complete degeneration of the organ of Corti using specific markers of leukocytes (anti-CD45 and anti-ED1 antibodies).
Very few macrophages were seen in the cochleas of the non-treated rats. In the amikacin damaged organs of Corti, a peak in macrophage density (around 20 fold the normal value) occurred during the week post-treatment when hair cells died through apoptosis. Surprisingly, after complete hair cell disappearance, the density of phagocytes remained very high during one additional month coinciding with the progressive degeneration of the remaining non-sensory epithelial cells. Density of phagocytes did not return to normal value even after 10 weeks (almost 5 fold normal values).
These results suggest that macrophages mediate clearance of the hair cell apoptotic corpses but a subsequent chronic inflammation may contribute to induce secondary degradation of the supporting cells.

P51

Noise induced hearing loss (NIHL) and vestibular functional damage

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The most common reason for sensory-neural hearing loss is degeneration of cochlear sensory (hair) cells, resulting from ototoxic drugs, autoimmune diseases, aging and over-stimulation. Specifically, exposure to intense sound induces not only severe hearing loss but also vestibular deficits at times, however, only sub-clinical. Most researches, have analysed noise induced hearing loss NIHL; on the contrary, impairment of the vestibular function has not been systematically addressed and the different vulnerability of the two systems has not been compared. The present research addresses acoustic trauma in the guinea pig; noise exposure was developed and in parallel the time course of both acoustic and vestibular impairment was analyzed to define the different vulnerability of the two systems. Acoustic trauma was induced by a continuous pure tone of 6 kHz. The animals were anaesthetized and exposed for 60 minutes to 120 dB SPL. Auditory and vestibular functions were evaluated before the trauma, six hours after, and in days 1, 2, 3, 7, 14 21. Auditory function was evaluated by recording the ABR at 2-20 kHz. Vestibular function was evaluated by recording the vestibulo-ocular reflex (VOR). The animals underwent sinusoidal oscillations in the dark about their vertical and longitudinal axes to evoke horizontal and vertical eye responses. Table movements were measured by a servo-potentiometer and left eye movements by the infrared light projection technique. After 21th day morphological changes were analyzed. Preliminary data underline the analogy between the acoustic and vestibular damage. The time course paralleled as regards both the temporary and permanent damages however the VOR gain loss appeared less relevant.

P52

The vestibular toxicity of cis-crotononitrile does not require CYP2E1-mediated metabolism

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Several nitriles cause hair cell degeneration in rodents. The molecular basis of this effect remains to be elucidated, including the question of the final toxic compound, i.e., whether the parent nitriles or unknown metabolites are responsible for the inner ear effect. cis-Crotononitrile (cis-2-butenenitrile) is a good candidate to address this question, because it is structurally simple and because it shows isomeric specificity (the trans-isomer is not vestibulotoxic). It is known that some related nitriles are metabolized by CYP2E1, generating an epoxy metabolite then breaking down to cyanide. The present work was designed to test the hypothesis that this metabolic pathway is responsible for the vestibular toxicity of cis-crotononitrile, by using a K.O. strain of mice for this enzyme (CYP2E1-/-). Wild type control (129S1) and CYP2E1-/- mice were used in basal condition or following exposure to 1 % acetone in the drinking water for one week; this procedure enhances the expression of the cyp2e1 gene. These mice were then administered cis-crotononitrile (0, 2, 2.25 and 2.5 mmol/kg, oral, n=6-7/group), and observed for mortality, for behavioral indices of vestibular dysfunction up to day 21 post-dosing, and for hair cell loss by scanning electron microscopy at 4-8 weeks. Acetone exposure enhanced the lethal effects of cis-crotononitrile in 129S1 but not CYP2E1-/- mice. However, CYP2E1-/- mice showed a similar or even higher susceptibility than 129S1 mice to vestibular toxicity, as indicated by either behavioral or histological methods, and this was not modified by acetone pre-treatment. The results demonstrate that CYP2E1 is not required for the vestibular toxicity of cis-crotononitrile, and suggests that neither cis-2, 3-epoxy-butironitrile nor cyanide are the ultimate toxic agents.

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P53

Identification of markers for neuronal plasticity in the auditory system

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Aberrant neuronal activity is known to lead to changes in neuronal plasticity. However, molecular changes following sensory trauma and the subsequent response of the central nervous system are only poorly understood. We focused on finding a molecular tool for monitoring the features of excitability which occur following acoustic trauma to the auditory system. Of particular interest are genes that alter their expression pattern during activity-induced changes in synaptic efficacy and plasticity. The expression of brain-derived neurotrophic factor (BDNF), the activity-dependent cytoskeletal protein (Arg3.1/arc), and the immediate early gene c-Fos were monitored in the peripheral and central auditory system hours and days following a traumatic acoustic stimulus that induced not only hearing loss but also phantom auditory perception (tinnitus), as shown in rodent animal behavior models. A reciprocal responsiveness of activity-dependent genes became evident between the cochlea and the primary auditory cortex (AI): as c-Fos and BDNF exon IV expression was increased in spiral ganglion neurons, Arg3.1/arc and (later on) BDNF exon IV expression was reduced in AI. Expression of Arg3.1/arc is changed in layer II of the AI with a reduction in the tonotopic area representing lower frequencies (10 kHz). We extended our studies to the transmembrane proteins neurexin and neuroligin, to examine whether these genes are involved in pre- and postsynaptic activity changes and therefore also mirror trauma-induced changes in synaptic responsiveness. The data are discussed in the context of using activity-dependent genes to monitor trauma induced plasticity changes in the auditory system.

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P54

No difference between inner and outer hair cells in uptake of FM1-43

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It is generally found that outer hair cells (OHC) are more sensitive to aminoglycoside antibiotics than inner hair cells (IHC). In mice, a single injection of kanamycin followed by a single injection of the loop diuretic bumetanide results in rapid loss of all OHC, but IHC may persist for several weeks. One reason for the differential vulnerability maybe differential uptake of the aminoglycoside. It has been suggested that both the fluorescent styryl dye FM1-43 and aminoglycoside antibiotics enter hair cells via the transduction channel. Thus, FM1-43 might be used to trace potential uptake of aminoglycoside. Auditory bullae from mice were isolated, the cochleae rapidly exposed and bone at the apical end of the cochlea removed to expose the apical turns of the organ of Corti. The cochleae were incubated in FM1-43 in HEPES buffer at 37℃ for 10 secs, transferred to ice-cold HEPES, and after washing in HEPES, strips of organ of Corti were isolated and examined as whole mounts. IHC and OHC showed similar levels of dye uptake. FM1-43 was also administered systemically to mice via a subcutaneous injection. Some animals then received an intraperitoneal injection of bumetanide, other animals did not. Examination at various time points following injection revealed IHCs labelled at least as intensely as OHC, and that bumetanide promoted labelling of hair cells: in animals that received the diuretic cochlear hair cells were labelled by 6 hours post-injection but in mice which were not injected with diuretic labelling at this time was minimal. The kanamycin-bumetanide protocol does not cause loss of hair cells from the vestibular system and in animals which received FM1-43 and diuretic vestibular hair cells were not labelled suggesting FM1-43 indeed may indicate aminoglycoside uptake. If so, these results suggest that in the mature organ of Corti the differential sensitivity of IHC and OHC is not due to differences in drug uptake.

P55

Up-regulation of notch signaling following ototoxic deafening

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During development, the mammalian cochlea is specified into a mosaic of heterogeneous cell types under the direction of Notch signaling system. Initially, Notch activity confers a prosensory character on groups of cells by lateral induction; subsequently, it is involved in the establishment of specific patterns of hair cells and supporting cells by lateral inhibition. Non-mammalian vertebrates are capable of regenerating hair cells following ototoxicity. In the chick, the regenerative process includes increases in Delta-Notch signaling. Such changes have not been reported in the mammalian ear. We tested whether differences in Notch expression (in comparison to birds) following ototoxicity are responsible for mammals' inability to produce hair cells postembryonically. Pigmented adult male guinea pigs were deafened systemically with kanamycin and ethacrynic acid, and then sacrificed either 24 hours, 3 days, 5 days, or 7 days later. Immunohistochemistry of cochlear whole mounts were performed using primary antibodies to Notch1, Jag1, Delta1, and activated Notch1 (NICD). Results showed an up-regulation of Notch1 receptor expression in inner hair cells at 1-day, which was stable until 7-days. Jag1 expression was up-regulated in the Pillars and Deiters cells; expression peaked at 1 day and gradually decreased over the remaining time periods examined. NICD expression was widespread and peaked after 1-day post deafening, slightly decreasing at 3-days and remaining stable until 7-days. Delta1 expression during all time points was nonexistent. Therefore, we conclude that while in birds, the pro-hair cell ligand Delta1 is activated following ototoxicity, whereas in mammals the pro-supporting cell ligand Jag1 is activated. This reduces and impedes the chances of pre-existing progenitors in the organ of Corti from spontaneously differentiating into hair cells following ototoxic insults and subsequent Notch activation.

P56

Middle ear gas loss in inflammatory conditions: the role of mucosa thickness and blood flow

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Several middle ear (ME) pathologies are associated with ME gas deficit. These include in particular the chronic otitis media diseases that are associated with inflammation (hence, increased ME mucosal blood flow) and/or reduced Eustachian tube (ET) function.
The present study models the trans-mucosal gas exchange in normal and inflamed middle ears of rats. The model evaluates the role of the gas diffusion path in the ME mucosa using mucosa thickness as its index and the role of the mucosal blood flow rate on ME gas economy in order to compare between normal and inflamed MEs.
An experimental method employing ME gas volume changes at constant pressure due to trans-mucosal gas exchange, and blood gas values from the literature, was used in anaesthetized rats to corroborate the model. Mucosa thickness was measured as an index of the gas diffusion path between the ME space and the ME circulation. ME inner surface area was estimated from its measured gas volume. Inflammation was inflicted by applying lipopolysaccharide (LPS) into one ear. The contralateral ear served as control.
ME gas volume decreased significantly faster with time ($p=0.02$) in inflamed ears (0.107µL/min ± 0.034SD, n=10) vs. control ears (0.067µL/min) ± 0.036SD, n=10). Mucosa thickness was significantly thicker in inflamed ears (48.4µm ± 11.0SD) vs. controls (20.5µm ± 10.1SD).
The mathematical model, the experimental results, and the blood gas values were used to estimate the relative effective mucosal blood flow rate. The model predicts that in spite of almost doubling mucosa thickness in LPS treated ears, the increased gas loss in inflamed ears may be explained by increased mucosal blood flow rate.
We suggest that the ability to estimate ME blood flow as obtained by applying the model to the measurements, is relevant to medical management of inflamed ME.

P57

Cubilin and megalin co-localize in the inner ear

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Cubilin and megalin are two multifunctional endocytic receptors expressed in many absorptive epithelia of the organism. These receptors, although serving independent functions as well, act together in concert to perform significant physiological functions in several tissues.
The aim of the present study was to investigate the expression of cubilin and megalin in the inner ear of neonatal rats. Furthermore, we investigated the binding properties of six different aminoglycosides (AG) to cubilin and compared the results to data obtained for megalin, since megalin acts as a drug receptor for AG and other well-known ototoxic drugs. In the cochlea, immunohistochemical labelling for cubilin showed expression corresponding to the apical surface of the strial marginal cells, epithelial cells at the spiral prominence and epithelial cells of Reissner's membrane facing the cochlear duct. In the vestibular apparatus, labelling was found in dark cells of the utricle and those flanking the crista ampullaris of the semicircular canals. The exact same tissue distribution was found for megalin. Specific antibody reactivity and receptor tissue distribution was confirmed by immunoblotting. Our results thus demonstrate that cubilin co-localizes with megalin in the inner ear. These findings support the prevailing view that cubilin and megalin comprise a dual-receptor complex facilitating the function of each other. The physiological role of this dual-receptor complex in the inner ear remains unclear. However, the tissue distribution of the receptors combined with their multi-ligand binding properties could indicate, that these receptors act as a high capacity – low affinity system for scavenging of macromolecules from the endolymph. The receptor-ligand interaction analysis showed that all six AG bind to both cubilin and megalin and with approximately the same affinity. These results demonstrate a novel role for cubilin as a drug receptor for AG and possibly other ototoxic substances.

P58

Neuronal activity in the inferior colliculus and auditory cortex in rats with noise-induced altered gap detection ability

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Loud noise exposure not only damages the structures of the inner ear, but may also specifically affect the function of the central part of the auditory system. We have previously demonstrated enhanced middle latency cortical evoked response (MLR) amplitudes in rats after noise exposure (1). Simultaneously, the gap detection threshold (GDT) was shown to be increased after noise exposure, indicating the presence of tinnitus in noise-exposed rats (2). Several studies have linked experimentally induced tinnitus to increases in the spontaneous activity of neurons in the central auditory nuclei. The aim of our work was to analyze spontaneous and evoked neuronal activity in the inferior colliculus (IC) and auditory cortex (AC) in rats exposed to noise and to compare it with previously assessed hearing thresholds, GDT values and MLR amplitudes. Neuronal activity was evaluated in rats 1-2 months after noise exposure (broad-band noise, 123 dB SPL, 1 h). Extracellular single-unit or multiple-unit activity was recorded in the IC and AC with a 16-channel electrode probe; in each neuron spontaneous activity, the responses to broad-band noise stimuli and the tuning curve were evaluated as well. The average level of spontaneous activity in noise-exposed rats was 2-5 times higher in both the IC and AC as compared with control unexposed rats. The level of the spontaneous activity was increased in the majority of neurons independently of their characteristic frequency. The study presents a simple model that enables the correlation of recorded electrophysiological data with the behavioral gap detection ability of the animal. References: (1) Syka J., Rybalko N.: Hear. Res. 139: 59-68, 2000. (2) Rybalko N., Syka J.: Hear.Res. 200, 63-72, 2005.

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P59

Meniere's disease and the electromodel

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The concept of electroreception by the inner hair cells (IHC) was first published in 1970 and expanded by Offutt in1984. By applying the principles of the electromodel, we may now be able to understand many factors that result in many hearing dysfunctions. It is now apparent that the integrity of Reissner's membrane is a critical concern for preventing Menière's Disease from going into its final stage. The integrity of the endolymph and the potassium concentration is critical for the electroreception by the IHC. The IHC are stimulated by the piezoelectric potentials from the tectorial membrane or the cochlear microphonics from the outer hair cells. At high stimulus levels and when there is no electroreception, IHC are sensitive to mechanical stimuli.
Due to repeated damage by hydrops, the integrity of the Reissner's Membrane is lost in stage two of Menière's Disease. This results in the loss of the potassium concentration, the cessation of electroreception by the inner hair cells and a high, flat threshold curve. The Meniett or P100 may be useful only during the first stage to purge excess endolymph and prevent the loss of the integrity of Reissner's Membrane.

Gene

P60-P62

P60

Association of SNPs in KCNE1 and KCNE3 genes with Meniere's disease

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KCNE potassium channels associated with KCNQ potassium channels are known to play an essential role in trans-epithelial ion and water transports in several organs including heart, kidney, colon, and small intestine. In the inner ear, the expression of KCNE1 channel has been confirmed in the stria vascularis and null mutation of this gene causes an abnormal development of the endolymphatic system in the mouse cochlea accompanied by severe deafness. Mutations in KCNE1 gene have been linked to some forms of cardiac arrhythmia, including Jervell and Lange-Nielsen syndrome with severe congenital deafness.
RT-PCR amplification and direct sequencing confirmed the dominant expression of KCNE1 mRNA in the rat cochlea and that of KCNE3 mRNA in the endolymphatic sac. IH and ISH studies could demonstrate that KCNE1 channel was mainly expressed in the the stria vascularis while KCNE3 channel was intensely expressed in the epithelium of the endolymphatic sac. These results indicate that KCNE channels should be active in trans-membrane ion and water transports in the inner ear.
Although the bases for both the sporadic and inherited forms of Meniere's disease (MD) remain undefined, it is likely to be multi-factorial, one of the factors being a genetic predisposition. Recently, genetic association studies on complex disease have become very popular and most of them are case-control studies using single nucleotide polymorphisms (SNPs) as markers. In the present study, we have conducted the genetic association study with optimized sampling, optimized phenotyping/genotyping, and a selection of KCNE genes as the candidate genes. The SNPs analyses identified 112G/A SNP in KCNE1 gene and 198T/C SNP in KCNE3 gene. For both KCNE1 and KCNE3 genes, a significant difference in frequency of each SNP was confirmed between MD cases and non-MD control subjects. The result indicates that SNPs in KCNE1 and KCNE3 genes might determine an increased susceptibility to develop MD.

P61

KCNQ4, a gene for age related hearing impairment

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Age-related hearing impairment (ARHI) is the most common sensory impairment among the elderly. It is a complex disorder influenced by genetic as well as environmental factors. Single Nucleotide Polymorphisms (SNPs) in a candidate susceptibility gene, KCNQ4, were examined in two random, independent Caucasian populations. Two QTLs were investigated: Zhigh and Zlow, a measure of high, respectively low frequency hearing loss. In the first population, the statistical analysis of 23 genotyped SNPs spread across KCNQ4 resulted in significant p-values for 2 SNPs (SNP9 and SNP15) for Zhigh and 4 SNPs (SNP12, SNP14, SNP17 and SNP18) for Zlow. The linkage disequilibrium (LD) structure of KCNQ4 was subsequently determined in a 34 kb-region surrounding the significant SNPs, resulting in 3 LD-blocks. LD-block 1 contains SNP9 and covers an area of 5 kb, LD-block 2 measures 5 kb and surrounds SNP13 to SNP18 and LD-block 3 spans 7 kb. Five tag-SNPs of block 1 and 2, and 2 positively associated SNPs were subsequently genotyped in the second population. Again, several SNPs were positively associated with ARHI: 1 SNP (SNP18) for the high frequencies and 2 SNPs (SNP9 and SNP12) for the low frequencies, although only a single SNP (SNP12) resulted in significant p-values in both populations. Nevertheless, the associated SNPs of both populations were all located in the same 13 kb region in the middle of the KCNQ4 gene. Possibly the causative SNP might be located within this region. Future association and functional studies of KCNQ4 will contribute to the identification of the causative SNP for ARHI.

P62

Auditory neuropathy in a hereditary mitochondrial disease: evaluation of hearing function in Friedreich ataxia mouse transgenic model

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Friedreich ataxia (FRDA), the most common form of inherited ataxia, resulting from a deficit in mitochondrial protein (frataxine), implying an excessive vulnerability of neurons to oxidative stress, results in a variety of neurological disorders. Hearing abilities can be impaired: some patients, notably among those with more severe neurological symptoms, complain about auditory difficulties or decreased intelligibility in noise.
In our preliminary clinical study, the auditory disorder of FRDA matches the definition of auditory neuropathy (identified by degradation or absence of auditory brainstem responses and presence of evoked acoustic otoemissions). FRDA's neuropathy appears with prolonged acoustic stimulation. This is consistent with the involvement of mitochondrial dysfunction and energetic deficit in FRDA, in view of the high density of mitochondria in auditory neurons.
The goal of this study is to evaluate the consequence of frataxin impairment on the hearing function in a neural knock-out model of the FRDA.
We determined the consequence of frataxin deficiency on the auditory nerve physiology (using auditory brainstem-evoked responses- ABR) and on cochlear hair cells (mainly the outer hair cells, using distortion product otoacoustic emissions – DPgram) at various stages (mutant mice without neurological symptoms [20 weeks of age] and with neurological symptoms [30 & 40 weeks]).
If it turns out that this animal model presents an auditory dysfunction similar to the human one, it will be interesting to evaluate the possible effects of an antioxidant treatment on cochlear and auditory neural functions.

Protection, Repair and Regeneration

P63-P74

P63

Protective effect of Ebselen on noise induced cochlear damage in rat

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Background and Objectives: With the advancement of modern civilization and mechanical development of society, the prevalence of noise-induced hearing loss is increasing. There were some suggestions that noise-induced hearing loss may be reduced or prevented with antioxidant treatment. The purpose of this study is to evaluate the effect of ebselen as a free radical scavenger or antioxidant in noise-induced cochlear damage. Materials and Method: Thirty male Sprague Dawley rats (250-300 g) with normal auditory brainstem response (ABR) thresholds were exposed for 6 h to 115dB SPL broad band noise. 10mg/kg ebselen were injected intraperitoneally at 12h before and 1h before noise exposure. After noise exposure, auditory brainstem response thresholds shift were evaluated. And a study for iNOS and nitrotyrosine expressions in cochlea were examined by immunohistochemical staining. Results: After noise exposure, auditory brainstem responses indicated that ebselen treatment reduced threshold shifts significantly. The expression of iNOS and nitrotyrosine were observed in hair cells, supporting cells of the organ of Corti, stria vascularis and spiral ganglion. And the expression of iNOS and nitrotyrosine were reduced in ebselen treated group compared with in non-treated group. Conclusion: Ebselen protects cochlea from noise by playing a role as a scavenger of reactive free radicals.

P64

TEM analysis of the synaptic consequences of hair cell loss and regeneration in a bird with progressive, genetic inner ear abnormality

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Belgian Waterslager canaries (BWS) have a severe high frequency hearing loss accompanied by significant hair cell loss but relatively insignificant loss of auditory nerve fibers (Gleich et al 2001). Hair cell regeneration is on-going but does not fully restore hair cell number (Ryals and Dooling 2002). Since more hair cells are lost than neural fibers it may be that remaining BWS hair cells have a greater afferent synaptic density than normal. The purpose of the present study was to: 1) quantify synaptic density for hair cells and 2) report qualitative differences in auditory neural fibers and synaptic appearance in BWS and non-BWS. Preliminary TEM analysis in the middle and distal portions of the adult BWS BP shows that the number of afferent synapses per hair cell in BWS canaries decreases from neural to abneural edge in a manner similar to non-BWS. There is more variability in the number of afferent terminals per hair cell and the ratio of afferent terminals per hair cell is lower than non-BWS (Ryals et al 2005; Ryals et al 2006). Some BWS hair cells have multiple synaptic bodies in the absence of neural terminal contacts reflecting immaturity and ongoing neural activity. Efferent synaptic contacts are more abundant on BWS hair cells in the middle of the BP than in non-BWS. Some efferent terminals have two distinct appearances, reflecting differences in cytoplasmic matrix and vesicular packing, and suggesting that there may be two types of efferent synaptic endings on individual hair cells. We are currently analyzing over 200 TEM serial sections from the basal tip in BWS and non-BWS adult canaries.

These results offer the first direct measurements of synaptic density on hair cells in normal and genetically abnormal canary inner ear and provide a first step toward understanding the synaptic consequences of hair cell loss and regeneration in a bird with progressive, genetic inner ear abnormality.

P65

Protective properties of idebenone and vitamin E in noise induced hearing loss in the guinea pigs.

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Acoustic trauma oxidative stress associated with increased reactive oxygen species (ROS) is involved in the cascade of cochlear events that induce an apoptotic response. Recent studies suggest that mithocondrial pathway play a pivotal role in initiating apoptosis in cochlear hair cells and that antioxidant drugs represent a rationale for exploring therapeutic strategies in humans. This study examines whether the apoptotic cascade could be inhibited through two antioxidant molecules, idebenone and vitamin E attenuating the NIHL. NIHL was induced in Hartley albino guinea pigs by a continuous pure tone (6 kHz, 120 dB SPL, 40 min) in control group (I, n=6) or in treated animals. Guinea pigs were injected one-hour before noise exposure and once daily for the following three days with idebenone (group II, n=6), Vitamin E (group III, n=6)) or both idebenone plus vitamin E (group IV, n=6). Changes in cochlear function were characterized by means of ABR threshold shifts. Missing and apoptotic cells were identified with scanning electron microscopy (SEM) and terminal deoxynucleotidyl tranferase-mediated dUTP nick end labelling assay (TUNEL assay). One hour after noise exposure pretreatment with idebenone was most effective in reducing noise induced threshold shift at all frequencies ($p<0.05$). At this time, vitamin E causes a smaller decrease in threshold shift. In idebenone treated animals a slower decrease of threshold shift was observed in the following 3 weeks. In contrast vitamin E caused a significant reduction in the induced threshold shift at all frequencies at 7 days after treatment. Treatment before noise exposure with either idebenone or vitamin E decreased the number of apoptotic cells and significantly reduced the cochlear outer cell loss. These results indicate that both idebenone and vitamin E decrease noise induced ABR threshold shift and attenuate noise induced OHC loss supporting the notion that ROS are involved in NIHL.

P66

Pharmacological protection against cisplatin induced ototoxicity.

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Cisplatin (CDDP) is recognized as one of the most ototoxic drugs used in clinical applications. Previous studies in our lab have suggested that the Sprague Dawley rat is one of the best animal models to study the effects of CDDP on the inner ear. In this study we have evaluated the pharmacological protection given by two different aminoacids: D-methionine (D-met) and L-N-acetylcysteine (L-nac). 16 mg/kg CDDP was administered intraperitoneally at the beginning of the experiment. The animals were divided in 3 groups: group 1 (n = 16) received D-met (300 mg/kg) as protective molecule, group B (n = 16) received L-nac (350 mg/kg) and group C (n = 4) served as the control group and received an equal volume of saline solution. Hearing function was assessed by the use of auditory brainstem responses (ABR), distorsion product otoacoustic emissions (DPOAE) and transient evoked otoacoustic emissions (TEOAE). The hearing status of the animals was evaluated at 3 different time points: before the CDDP administration, and then at 72 and 168 hours from the CDDP treatment.
Different hearing patterns were found between the 3 groups: (i) a marginally better protection was found for the D-met treated group at 72 and 168 hours, ($P < 0.04$, and $P< 0.035$ respectively) in comparison to the other treated group; (ii) the L-nac treated group showed a moderate hearing protection in comparison to the control group at 168 hours; (iii) the animals of the control group presented irreversible hearing deficits at all tested frequencies which was consistent with histological analysis.
In addition the electrophysiological data obtained in these experiments were used to establish a mathematical model correlating DPOAEs and ABR values, in order to have faster hearing checking protocols for future experimental ototoxicity set-ups.

P67

Ethanolic extracts from Hemidesmus indicus Linn. display otoprotectant activities on organotypic cultures

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Aminoglycoside antibiotics such as gentamicin represent one of the most widely used class of compounds in the world.At the basis of the success of these antibiotics there are two important factors the effectiveness as antimicrobials and the low cost.Given this potentially heavy social impact in the use of aminoglycoside antibiotics, it is important to discover ways of reducing or eliminating their side effects. A potentially useful resource can come from the use of plant derivatives used in traditional medicine from developing countries. In particular, among other medicinal plants, Hemidescus indicus has been reported to exhibit biological activity, either anti-tumor, anti-inflammatorily and anti-microbial activities. On the other hand the molecular basis of aminoglycosides toxicity is not well understood yet. In the present report we present data on the use of alcoholic extract from the roots of Hemidesmus indicus to prevent the GM-induced loss of hair cell. To test for this activity, we used organotypic cultures of organs of Corti from postnatal rats, which have been previously employed as an ex vivo model to study the effects of various damaging stimuli. In this model, we evaluated both the safety and otoprotective range of action of Hemidescus indicus extract, and compared the effect on GM uptake of different plant extracts.

P68

Quantitative analysis of surviving spiral ganglion cells in BDNF-treated cochleas of deafened guinea pigs

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Electrical stimulation and application of exogenous neurotrophins can enhance spiral ganglion cell (SGC) survival in deafened animals. Shepherd et al. (2005) have also observed that average SGC size in cochleas treated with brain-derived neurotrophic factor (BDNF) is larger than in non-treated deafened cochleas. In this study, we have performed quantitative analyses of the SGCs at the light-microscopical (packing densities) and ultrastructural level (soma area, nuclear area) in deafened guinea pigs after delayed neurotrophic treatment.
Guinea pigs were deafened by means of co-administration of kanamycin and furosemide. The functional effect of the deafening technique was confirmed by measuring the compound action potentials (CAPs) and auditory brainstem responses (ABRs), at frequencies from 2-16 kHz. Two weeks after deafening, the right cochleas were implanted with an electrode and cannula, which was attached to an Alzet mini-osmotic pump filled with BDNF (100 ug/ml). BDNF was infused into the cochlea over a period of four weeks. Left cochleas were not treated with BDNF and served as controls. Six weeks after deafening, both left and right cochleas were fixed and processed for ultrastuctural examination and quantitative analysis. Data were compared with those obtained from non-deafened animals.
SGC packing densities in the right (BDNF-treated) cochleas were 2-3 times higher than in the left (non-treated deafened) cochleas. In four out of six animals, BDNF treatment (right cochleas) resulted in a significant increase in the average SGC soma area as compared to non-treated deafened (left) cochleas. These data confirm Shepherd's findings.

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P69

Effects of combined electrical stimulation and GDNF treatment on spiral ganglion cells in deafened guinea pigs

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Sensory-neural hearing loss leads to degeneration of spiral ganglion cells (SGCs). For the results with a Cochlea-Implant (CI) the SGC-density plays a decisive roll. Several studies indicate that SGC degeneration can be reduced by electrical stimulation (ES). Glial cell line-derived neurotrophic factor (GDNF) treatment leads to a decrease in SGC loss, too. The aim of this study was to investigate if both interventions have a synergistic effect.
Pigmented guinea pigs (n = 24) were systemically deafened by a co-administration of kanamycin and ethacrynic acid. Three weeks after deafening, the left cochleae were implanted with an electrode/cannula device. The cannula was attached to a mini-osmotic pump (flow rate: 0.5µl/h) filled with either GDNF (100ng/ml) or artificial perilymph (AP). Two groups of animals received GDNF and two AP. One group getting GDNF and one group getting AP were additionally electrically stimulated via a portable stimulator for 24 days, 24h a day. 48 days after deafening the cochleae were extracted and prepared for histology. The outline of each Rosenthal´s canal profile was traced and all SGCs in this area were counted to generate a SGC-density (cells per 10.000µm^2).
Our results demonstrate that ES or GDNF treatment alone caused a significant SGC survival when compared to the control group. The GDNF treatment led to a slightly but not significantly higher SGC survival compared to the single ES treatment. The combination of GDNF and ES resulted in a sixfold higher SGC density ($p < 0,001$) compared to controls. GDNF+ES led to a more than three times higher density of protected SGCs compared to the ES treatment ($p < 0,01$) and approximately to a 2.2 times higher density compared with the effect of GDNF alone ($p < 0,05$).
We conclude that the combination of local intracochlear delivery of 100ng/ml GDNF and simultaneous electrical stimulation of the inner ear has more potential for SGC-protection than one of these interventions alone.

P70

Mitochondrial regulation of damage-induced intercellular Ca^{2+} waves in cochlea supporting cells in vitro

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Sensory cell damage triggers changes in cytosolic calcium ($[Ca^{2+}]c$) in the surrounding support cells. Damage-induced changes in $[Ca^{2+}]c$ propagate as an intercellular wave through cochlea supporting cells. The mechanism involves an IP3-dependent regenerative Ca^{2+} wave elicited in response to extracellular ATP It is well documented in numerous cell types, that regulation of $[Ca^{2+}]c$ can alter the properties of Ca^{2+} wave propagation. $[Ca^{2+}]c$ can be regulated by a number of different mechanisms including endoplasmic reticulum and plasma membrane Ca^{2+}-ATPase activity and the mitochondrial Ca^{2+} uptake pathway. Mitochondria in particular have been shown to modulate intracellular wave propagation in oligodendrocytes, astrocytes, neurons and oocytes by acting as high capacity fixed Ca^{2+} buffers. Using digital fluorescence imaging techniques we have investigated whether mitochondrial Ca^{2+} uptake modulates damage-induced Ca^{2+} signalling in neonatal rat cochlea cultures. Mitochondria sequester Ca^{2+} via a Ca^{2+}-specific uniporter situated on their inner membrane. Using Rhod2, a fluorescent indicator of mitochondrial Ca2+ we confirmed that 80 µM Ru360 (a mitochondrial Ca^{2+} uniporter inhibitor) was effective in the cochlear cultures. Subsequent experiments using Fura 2 to measure $[Ca^{2+}]c$ revealed a significant contribution of mitochondria to damage-induced Ca^{2+} signalling in the cochlea. Blocking mitochondrial Ca^{2+} uptake enhanced the peak $[Ca^{2+}]c$ changes in both outer sulcus (Claudius) cells and Deiter's cell. In addition, in the presence of Ru360 the velocity of the damage-induced Ca^{2+} wave was increased by 24% in the outer sulcus/Claudius cell region. We are continuing to study the role of mitochondrial Ca^{2+} buffering in inner earl. For now, we conclude that mitochondria can modulate the spatio-temporal properties of damage-induced Ca^{2+} waves in the cochlea and further more the degree of mitochondrial modulation may differ between cochlea cell types.

P71

Protection against kanamycin-induced ototoxicity by kallidinogenase via bradykinin-B2 receptor in the rat cochlea

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It is still important to prevent and reduce aminoglycoside ototoxicity because aminoglycoside remain to be one of the first-line antibiotics in gram-negative infections and the intratympanic gentamycin treatment for intractable Meniere's disease is becoming increasingly popular. Since bradykinin is known to show a protective action against glutamate neurotoxicity in the retina and some parts of aminoglycoside ototoxity might involve glutamate neurotoxicity through an upregulation of glutamate receptors, we investigated the effect of bradykinin on kanamycin-induced ototoxity in the rat cochlea with a systemic application of kallidinogenase which cleaves tissue-ubiquitous kininogen into bradykinin.
Twelve rats were treated with a daily subcutaneous injection of kanamycin. The hearing thresholds were measured by ABR. In the protection experiment, seven rats were daily injected with kallidinogenase into the vein of tail together with kanamycin. Three rats were treated by venous injection of HOE140, which is a selective bradykinin-B2 receptor antagonist, 30 minutes before the application of kallidinogenase and kanamycin. Expression and localization of bradykinin-B2 receptor in the rat cochlea were examined by RT-PCR, ISH and IH.
RT-PCR analysis identified the expression of bradykinin-B2 receptor mRNA in the rat cochlea. IH study demonstrated that bradykinin-B2 receptor protein was mainly expressed in the spiral ganglion cells. Co-application of kallidinogenase together with kanamycin significantly reduced hearing impairments induced by kanamycin. Both outer hair cells and spiral ganglion cells were more preserved by an application of kallidinogenase. The protective effects were completely reversed by HOE140. These results suggest that kallidinogenase exerts a protective action against kanamycin-induced ototoxity through an activation of bradykinin-B2 receptor in the rat cochlea.

P72

Characterization of hair-cell regeneration in long-term cultures of post-natal rat utricles

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An in vitro model for long term culture (i.e. up to 28 days) of post-natal rat utricular maculae was established to characterize the renewal of mammalian vestibular hair-cells. An initial study by Berggren et al. (Hearing Research 2003, 180:115-125) demonstrated spontaneous renewal of hair cells following lesioning by gentamicin. A peak of stereocilliary bundle renewal occurred at three weeks post gentamicin exposure. In this work only the surface characteristics of the explanted macula epithelium was studied.

To study cellular events within utricular sensory epithelium during hair-cell renewal we repeated an experimental series that exactly mimicked our first study with the exception that the harvested explants were prepared for LM (plastic sections) and TEM analyses. Explants were serially sectioned and systematically analyzed with the aid of a computerized system regarding identification and quantification of cell types. One micron plastic LM sections chosen for TEM analysis were photo documented then re-embedded for TEM by using the method of King et al.(1982) and Pressen et al.(1996). Also, we have established a computerized method for volume measurements of these epithelia.

The present study verifies the stability and reproducibility of our utricular explant model. Hair cell and supporting cell density remained nearly intact up to 28 days in vitro in unexposed control explants. The gentamicin exposed explants showed a decline in hair cell density during the first 7 days followed by a recovery of hair cell density after 21 days post-exposure. Concomitant with cell counting the morphology of the sections was studied and we observed what appeared to be fused cells (i.e. a cell containing two nuclei) and also cells in mitosis.

These findings demonstrate a unique in vitro model for studying the renewal of hair cells that will be beneficial in determining mechanisms that underlie hair-cell regeneration.

P73

Overexpression of heat shock protein 70 inhibits neomycin-induced hair cell death

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Sensory hair cells are sensitive to death from noise trauma, aging, and some therapeutic drugs. Two classes of ototoxic drugs are the aminoglycoside antibiotics and the antineoplastic agent cisplatin. Exposure to these drugs results in hair cell death that is mediated by specific apoptotic proteins. Aminoglycoside-induced hair cell death is mediated by c-Jun N-terminal kinase (JNK) and caspases. The induction of heat shock proteins (HSPs) in response to cellular stress is a highly-conserved response that can promote cell survival in many systems by directly inhibiting apoptotic proteins, including JNK and caspases. We have previously shown that heat shock (43oC for 30 minutes) results in a robust upregulation of HSPs in the hair cells of the adult mouse utricle in vitro. In addition, heat shock results in significant inhibition of both cisplatin- and aminoglycoside-induced hair cell death. Since heat shock can result in upregulation of a number of stress-induced proteins, we have now begun to examine the role of a single HSP in this protective effect. The most strongly-induced HSP in our system is HSP-70, which is upregulated over 250-fold 2h after heat shock. In order to determine whether HSP-70 alone is sufficient to protect hair cells, we have utilized transgenic mice that constitutively overexpress rat HSP-70 under the control of the beta-actin promoter. Utricles from HSP-70 overexpressing mice and wild-type littermates were cultured in 2 mM neomycin for 24h. This neomycin exposure results in death of about 50% of the hair cells in the wild-type utricles. The HSP-70 overexpressing utricles were significantly protected against neomycin-induced hair cell death, losing only about 20% of their hair cells. This difference was statistically significant (two-way ANOVA, $p<0.05$). This protective effect was achieved without a heat shock. These data indicate that HSP-70 alone is sufficient to protect hair cells against neomycin-induced death.

P74

An animal model of cochlear implantation with perilymphatic drug delivery

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Drug delivery to the inner ear is an interesting mean of achieving high local doses while avoiding general side effects. It is also a promising approach to maintain the residual hearing during and after the cochlear implantation. Otoprotective components could be released through a microcatheter located inside the electrode carrier. The conception of an animal model is a first step to assess different molecules before human clinical trials.
Guinea pigs with normal hearing were implanted with a prototype electrode carrier (MED-EL) that comprised the electrode and a microcatheter that was connected to an osmotic minipump (Alzet). The tested drug was the antioxydant N-acetyl-cysteine; artificial perilymph was injected as control. Hearing thresholds were evaluated with auditory brainstem responses (clicks, tone bursts) before, immediately after the implantation, and at day 7 and day 30 postoperatively. Histological study of the cochlea was performed at day 30. Some animals had a CT-scan to check the implant position at day 30.
An immediate postoperative hearing loss of 34 ± 2.2 dB (click simulation, mean ± SEM, n=14) was observed. It remained stable at day 7 (-36 ± 5.2 dB, n=13), and at day 30 (-32 ± 2.9 dB, n=4). Preliminary analysis did not find any protective effect on hearing preservation from the N-acetyl-cysteine. The average loss in the control group was 33 dB ± 2.6 (n=10) and 34 ± 6.0 (n=3), at day 7. CT-scan images showed that implants were introduced in the basal turn at a depth angle varying from 270 to 360 degrees, and that the implants stayed in a good position during one month. Histological studies are being conducted.
In conclusion, this animal model is simple and reproducible. It mimics the cochlear trauma generated during cochlear implantation and would allow the evaluation of drugs to achieve a better preservation of residual hearing (e.g. antioxydants, corticoids, or growth factors).

Cell and Gene Therapy

P75-P80

P75

Bone marrow transplantation as a strategy for the treatment of autoimmune hearing loss in MRL/Mp-lpr/lpr mice

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Sensorineural hearing loss (SNHL) has been reported to develop as a main part of or in combination with systemic and organ-specific autoimmune diseases. The aim of the current study is to treat autoimmune SNHL in MRL/Mp-lpr/lpr (MRL/lpr) mice, a murine model of systemic autoimmune disease, using allogeneic bone marrow transplantation (BMT), which replaces recipient bone marrow cells with bone marrow cells from a non-autoimmune prone donor. The results indicate that BMT can be used to treat SNHL; cochlear pathology, serum autoantibodies and lupus nephritis are ameliorated. Therefore, it is conceivable that the autoimmune SNHL in the MRL/lpr mice results not from defects in the cochlea, including the stria vascularis, but from defects in the bone marrow, and BMT would therefore provide a curative effect on inner ear autoimmune dysfunction associated with systemic autoimmune diseases.

P76

Role of systemic immune system in survival of the spiral ganglion cells

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IDEA
A substrain of the senescence-accelerated mouse (SAM), the SAMP1 mouse, is an animal model for accelerated senescence including the age-related acceleration of both immunological dysfunction and hearing loss caused by the impairment of spiral ganglion cells. In the present study, we have examined the preventive effect of systemic immune functions reconstituted with allogeneic bone marrow cells on accelerated presbycusis in this mouse.
METHODS
Young SAMP1 mice were fatally irradiated and then reconstituted with bone marrow cells from GFP transgenic mice, which are introduced green fluorescence protein (GFP) DNA into C57BL/6 mice, low-presbycusis-prone strain mice.
RESULTS
Transplantation of bone marrow cells from GFP mice was found to prevent the development of both hearing loss (ABR with click sound or pure tone), and degeneration of spinal ganglion cells in SAMP1 mice.
Fluorescence study indicated that no donor cells including bone marrow cells and immunocompetent cells reach the spiral ganglion locally. Immunological assay indicated that age-related impairment of T cell function in SAMP1 mice were not observed in SAMP1 mice transplanted bone marrow cells from GFP mice, [GFP to SAMP1] mice.
CONCLUSION
The current study indicates that some types of accelerated presbycusis do not result from defects in the cochlea but do from defects in bone marrow cells. No donor cells were observed in the spiral ganglion of [GFP to SAMP1] mice, therefore, hemopoietic stem cells in the bone marrow or immunocompetent cells derived from those cells regenerate or maintain the spiral ganglion cells locally. Because bone marrow transplantation has prevented development of both hearing loss and T cell dysfunction, it is conceivable that systemic immune system including T cells participates in survival or maintenance of the spiral ganglion cells.

P77

Transfer of autoimmune inner ear disorder by bone marrow transplantation

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It has been reported that, in humans, autoimmune diseases such as autoimmune thyroiditis, Graves' disease, insulin-dependent diabetes mellitus and myasthenia gravis can be transferred to recipients who receive bone marrow from an affected individual. It has also been demonstrated that other autoimmune diseases such as psoriasis vulgaris, ulcerative colitis, and palmoplantar pustular psoriasis are resolved after the transfer of normal bone marrow into leukemia patients also suffering from autoimmune diseases. Based on these findings, we hypothesize that autoimmune diseases are bone marrow stem cell disorders that produce autoreactive immunocompetent cells including lymphocytes.
The MRL/lpr mouse, which is homozygous for the recessive lpr genes and has a mutation in the Fas gene encoding a cell-surface receptor for apoptosis, exhibits severe lymphadenopathy and develops SLE-like disease. It has recently been reported that this mouse also manifests sensorineural hearing loss (SHL) with cochlear pathology at 20 weeks of age. We examined the effects of reconstituting severe combined immunodeficient (SCID) mice with MRL/lpr bone marrow on the development of SHL in SCID mice, which normally develop neither SHL nor cochlear pathology. Immune-mediated SHL and cochlear pathology did, indeed, appear following transfer of MRL/lpr bone marrow into SCID mice. These findings suggest that the development of SHL and cochlear pathology observed in MRL/lpr mice and in SCID mice receiving MRL/lpr bone marrow are the result of bone marrow defects rather than the result of a problem intrinsic to the cochlea

P78

Relation between acceleration of age-related hearing loss and bone marrow cells from senescence-prone donors

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IDEA
There has been no effective therapy for age-related hearing loss, known as presbycusis and characterized by progressive deterioration of auditory sensitivity. Many factors have been proposed as contributing to this sensorineural hearing loss (SNHL), including genetics as well as diet, socioeconomics, and environmental variables. This study investigated the role of bone marrow cells (BMCs) and immunocompetent cells on auditory functions.
METHODS
BALB/c mice (2 months of age), non-presbycusis-prone mouse strain, were irradiated in fractionated doses. After the irradiation, the mice were transplanted with the whole BMCs (3 x 107) by bone marrow transplantation (BMT) from SAMP1 mice (2 months of age), presbycusis-prone mouse strain.
RESULTS
Acceleration of age-related hearing loss, early degeneration of spiral ganglion cells (SGCs) and impairment of immune function were observed in the recipient mice as well as in the SAMP1 mice. However, no spiral ganglion cells of donor (SAMP1) origin were detected in the recipient mice.
CONCLUSION
These results indicated that accelerated presbycusis, cochlear pathology, and immune dysfunction of SAMP1 mice can be transferred to BALB/c recipient mice using allogeneic bone marrow transplantation (BMT). However, although the BMCs themselves cannot differentiate into the spiral ganglion cells (SGCs), they indirectly cause the degeneration of the SGCs. Further studies into the relationship between the inner ear cells and BMCs are required.

P79

Transgenic cells expressing neurotrophic factors as a model for drug delivery into the inner ear

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It has been shown that the application of neurotrophic factors (NTF), e.g. GDNF, BDNF, NT3 increases the survival rate of spiral ganglion cells in-vitro and in-vivo. Fluid based delivery systems are mainly used for application of the NTF. Here, we present first approaches to use transfected cells for the production of NTF. If these cells could be attached to the surface of cochlear implants, this would provide a new possibility for drug delivery to the inner ear.
Murine Fibroblasts (NIH-3T3) were transfected via a lentiviral system to generate cell lines expressing several neurotrophic factors (GDNF, BDNF) and GFP under the control of a tetracyclin regulated promotor. The genes for the neurotrophic factors were arranged monocistronic or via an IRES-element bicistronic with GFP. As the bicistronic expression of GFP was insufficient we focussed on the monocistronic vectors for further experiments. The expressed and secreted neurotrophic factors were measured in the cell supernatant via an ELISA assay. Additionally we determined their biological activity by analyzing their potential to induce differentiation in pc-12 cells (rat pheochromocytoma cells). The supernatant of the GDNF producing cells was added to a growing culture of pc-12 cells and the induction of neurites was observed over a period of 10 days. Neurite outgrowth could be seen beginning from day 2. Most of the observed cells showed clear neurite outgrowth till day 10. No outgrowth could be observed when cells were cultured in medium without NTF.
In the following experimental phases transgenic fibroblasts will be cultivated on the surface of cochlear implant materials and the effect of the emitted NTF on spiral ganglion cells in co-culture will be analyzed.

P80

Approaches to non viral delivery to the inner ear

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Approaches of gene transfer to the inner ear have been mainly using viral vectors. Recent studies suggested that gene therapy through the transfer of math1 has potential to treat sensorineural hearing loss. Non viral gene therapy as a form of gene delivery may be ideal for the cochlea. However, cochlear non viral gene delivery has been examined by several studies and found to be inefficient compared to viral vector gene transfer. Two approaches to non viral delivery to the inner ear are here discussed. We investigated the polymer based delivery. Two separate entries to the inner ear were used: Vectors were delivered via application to the round window or delivery into the cochlea via a fenestration of the utricle.
Distribution of the vehicle was assessed in the cochlea as well as in the contra lateral ear and in the brain stem. Delivery to sensory hair cells and the spiral ganglion cells was seen in the cochlear and vestibular organ on both sides, suggesting that the vector spread through the cochlea aqueduct as previously demonstrated in viral vectors. We used fluorescence microscopy and transmission electron microscopy for detection.
Non viral gene transfer using advanced non viral vectors may provide a delivery system to the sensory hair cells, supporting cells and spiral ganglion cells.

Tinnitus

P81-P83

P81

Salicylate and pure tone trauma and band noise trauma induce tinnitus in a rat behavioural model

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Recently we could show the temporary presence of tinnitus in a rat behavioural model using high dosages of salicylate injections (350 mg/kg body weight, see also Rüttiger et al. 2003). As an alternative model we could demonstrate that also acoustic trauma of either pure tone (10 kHz, 118 dB SPL for 2 hours) or broadband noise stimuli (8-16 kHz band noise, 119 dB SPL, 2 h) led to permanent hearing loss and tinnitus, correlated with a differential change in the expression of activity dependent genes in cochlear spiral ganglion neurons, midbrain auditory nuclei and auditory cortical area AI.
The question was whether the tinnitus sensation induced by salicylate and noise trauma was founded on the same or different mechanisms. Rats were exposed to a traumatic acoustic stimulus and examined for temporary and permanent hearing loss and the development of tinnitus sensation. After evidence for a profound tinnitus manifestation, animals were injected with salicylate (350 mg/kg body weight) and retested for tinnitus sensation and general hearing function.
First results point to a non additive process of tinnitus generation and hearing impairment after combined salicylate and acoustic trauma treatment, suggesting similar mechanisms for tinnitus generation-most likely an impairment of adequate cochlear function.

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P82

Investigation of a BDNF missense variant in chronic tinnitus

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Objectives:
Neurotrophic factors play key roles in the developing auditory pathway including the sensory epithelium of the inner ear, and structures involved in the central nervous processing of auditory stimuli. In the present investigation, we explored a possible implication of variant BDNF in the susceptibility to chronic tinnitus.
Methods:
222 subjects complaining of chronic tinnitus were recruited from a tinnitus clinic and underwent detailed neuro-otological examinations including otoscopy, stapedius reflexes, middle ear pressure measurements, pure tone audiometry, tinnitus pitch and loudness matches. Subjects were genotyped for a biallelic BDNF missense variant (Val 66 Met). Prevalence of the substitution was compared to the prevalence in an ethnically homogenous group of healthy controls (N=317).
Results:
Carriers of the Met variant were significantly less likely to develop chronic tinnitus with comorbid hearing impairment ($p = .02$, OR = 1.62, 95% CI = 1.1-2.5). When no assumptions of dominance were made for the minor allele, the Met allele still conferred protection against tinnitus with hearing impairment ($p = .05$, OR = 1.42, 95% CI = 1.0-2.0).
Conclusions:
The present study suggests a role of variant BDNF in modulating the genetic susceptibility to chronic tinnitus with hearing impairment. Possible implications of this finding include a differential response to the pharmacological treatment of tinnitus, and specifically, to the neurotrophic effects of antidepressants.

P83

Reduction of Hearing Threshold after unilateral ear plugging for 10 hours

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Objectives: There is a large body of evidence that hearing loss is followed by changes in the central auditory system. Furthermore these neuroplastic changes are assumed to be strongly involved in the pathophysiology of tinnitus. In the present study we used psychophysical measures to explore, whether an experimental model of conductive hearing loss is able to induce adaptive changes of the auditory system within 10 hours.
Methods: 25 healthy young subjects (11 male, 14 female) with normal hearing function participated in the study. In all subjects the left ear was occluded with ear plugs for 10 hours providing an experimental model of conductive hearing loss. Pure tone audiometry of the left ear was performed at baseline, immediately after ear plugging as well as before and removal of the earplugs. Pure tone audiometry of the right ear was performed at baseline and after removal of the ear plugs.
Results: a significant reduction of the hearing threshold was observed in the left ear after 10 hours plugging. In the right ear no change of the hearing threshold was detected.
Conclusions: The observed changes of psychophysical measures in an experimental model of conductive hearing loss strongly suggest the occurrence of adaption processes of the auditory system within 10 hours. Further studies are necessary to identify structures and mechanisms involved and the possible role of these neuroplastic changes for the etiopathology of tinnitus.

Clinical Studies

P84-P94

P84

The design of Sara lip-reading test for Persian adults

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Objectives: developing a lip-reading test for Persian adults and standardization of the test in a group with normal hearing and vision.
Design: this study was a descriptive –analytic survey.
Subjects:1) 1000 simple, common, everyday sentences containing 26,668 phonemes selected from radio and television programs, telephone conversations and common everyday conversations;.2) 105 normally hearing and vision adults (59 females, 46males) ranged from 18 to 85 years old.
Main findings: 1) Frequency of occurrence of Persian phonemes (6 vowels, 23 consonants)
2) lip-reading, auditory-visual, frequency of occurrence, deaf, test.

P85

Benefits of cochlear implants in prelingually deafened adults

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A cochlear implant is an auditory prosthesis that, in many cases, can restore a useful level of hearing to individuals with severe-to-profound hearing loss. By employing direct electrical stimulation of the auditory nerve, a cochlear implant can substitute for non-functioning or undeveloped sensory structures in the cochlea and act as a transducer of sound information to the central nervous system. Cochlear implants have proved to be beneficial in children and postlingually deafened adults. On the other hand, results have not been as successful in the prelingually deafened population. A questionnaire was designed to acquire information about (1) implant and hearing-aid use, (2) educational, family, and occupational demographics, and (3) subjective ratings of quality of life and communication skills in prelingually deafened adult implant users. Results from 35 respondents showed that their assessments of everyday benefit from the implant were consistently high but unrelated to their speech-perception abilities. In addition, various demographic factors were not significant predictors of implant benefit. We therefore conclude that prelingually deafened adults can benefit significantly from cochlear implants and should not be automatically excluded from candidacy. Moreover, audiologists should become aware of the special rehabilitation needs associated with prelingually deafened adults who receive a cochlear implant.

P86

Peroperative NRT values in relation to rehabilitation after cochlear implant in children with the connexin 26 gene mutation

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Introduction: Mutations in the GJB2 gene are the most frequent cause aetiology of non-syndromal, prelingual deafness. The treatment of choice in patients with congenital prelingual deafness is cochlear implantation. Peroperative monitoring Neural Response Telemetry (AGF slope and NRT threshold levels) is essential in assessing proper functioning of the aural implant. Aim: The aim of this study was to ascertain whether language development differed in patients with varying aetiology of deafness after cochlear implantation. Methods: Nottingham scale and peroperative NRT measuring was used for the assessment. From the group of patients after cochlear implant surgery, performed in the ENT Clinic of the 2nd Medical Faculty, Charles University, Prague between the years 1999 and 2004, 35 patients were selected (15 with GJB 2 mutation, 20 without GJB 2 mutation). The results were evaluated using the Nottingham score at intervals of 6 and 24 months after implantation in all age groups. The correlation with this and peroperative NRT values was studied. Results: In patients without the mutation in GJB 2 gene, there was no relationship between the AGF slope and the outcome measured on the Nottingham scale, either half year or two years after the operation. In patients with the GJB 2 mutation we found correlation between the values of AGF slope and the outcome measured on the Nottingham scale, either half year or two years after the operation. Conclusion: In the group of patients with mutation of GJB 2 gene there is the correlation between the values of AGF slope and the outcomes measured on Nottingham scale. No such correlation exists for the values of NRT threshold. The prognostic value of the peroperative measure of AGF slope in the group of patients with GJB 2 gene mutation must be further investigated.

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P87

What is the impact of residual hearing as estimated by SSEP on the cochlear implant benefit?

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Introduction:Since 1999, the Steady State Evoked Potentials Method (SSEP) examined all candidates for cochlear implantation. Thanks to this technology, we are able to estimate the degree of residual hearing of frequencies between 0.5 kHz and 4.0 kHz. From July1, 1999 to May 31, 2006 were examined 2772 ears from 1391 patients with the SSEP method. The SSEP examination was conducted with ERA Systems equipment / Evoked Response Audiometer /,Model 10312. Ear phones were tube type, EAR Tone 3A 10 W. EEG pre-amplifier Opti –Amp 8000 was connected with optical cable. They were premedicated with chloralhydrat by rectum in the dose of 1 ml/kg. SSEP were recorded binaurally with carrier frequency at 500, 1000, 2000 and 4000 Hz amplitude modulated at 80 – 110 Hz.
Aim and Method: the study was designed to compare the results of rehabilitation of our patients with the results from other centers employing cochlear implant (CI).
We followed up the group of 45 CI users, operated from November 1, 1999 through May 31, 2002. The children were divided into 4 groups according to hearing threshold level. Two years after implantation, the patients' rehabilitation was evaluated using the Nottingham Scale of Speech Perception. The children reached 5-7 degrees on the Nottingham scale in all four groups. Because of the strict selection criteria in the group with higher degree of residual hearing, only 2 patients were evaluated.
Results: The results of the study are not in agreement with results from other centers. The patients that we observed were probably less differentiated from an audiologic point of view. For this reason, we didn't prove a relationship between cochlear implant benefit and the degree of residual hearing. On the other hand, our results show the great value of cochlear implantation for those children with no presence of residual hearing.

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P88

Heritability of age related hearing loss in Flemish families

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Several investigators studied the heritability of Age Related Hearing Impairment (ARHI). Gates et al. published a report on the Framingham study correlating hearing thresholds between family members, and Christensen et al. studied self reported reduced hearing in twins. The reports concluded that genetic factors play a role in age related hearing impairment. Gates et al. mentioned an increased correlation between female family members, more particularly in the low frequencies.
To confirm these findings we estimated the heritability of ARHI in 70 families.
70 families were collected in Flanders, most of then originating from around Antwerp. The inclusion criteria for the families were either 5 siblings between 50 and 75, or 4 siblings between 50 and 75 and at least one parent. Using a questionnaire the subjects were screened for other causes of hearing loss. All possible subjects (except the parents) underwent pure tone audiometry under standardised conditions.
Using the SAGE program we determined correlations between family members regarding hearing thresholds on all frequencies. The hearing thresholds levels were corrected for age and sex effects using a Z-score, based on the ISO 7029 standards.
In all available families, we studied the familial correlations between the siblings. Preliminary analysis of the familial correlations showed a tendency to higher correlations between sisters.
The findings published by Gates et al. showed a higher correlation between the hearing levels in sisters than correlations between brothers or brother-sister pairs. This indicates that the influence of environmental factors on the hearing thresholds is larger in males than in females. Early results of our study seem to confirm these findings. More complete results will be presented on the poster.

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Audiological characteristics of tinnitus in children and young adults

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There are many causes that can lead to tinnitus in children. The aim of this study was to evaluate the results of pure tone audiometry over an extended frequency range from 125 Hz to 16 kHz and recordings of otoacoustic emissions [transiently evoked otoacoustic emissions (TEOAE), spontaneous otoacoustic emissions (SOAE) and distorsion product otoacoustic emissions (DPOAE)] in fifty-two children and young adults, 6 – 25 years old, suffering from tinnitus of varying etiology. In 10 patients, tinnitus had started after noise exposure (most commonly after listening to loud music or following pyrotechnic accidents), in 9 patients tinnitus appeared after an infection (mostly viral), 8 patients suffered from tinnitus after head injuries, in 4 young adults tinnitus appeared in connection with frequent diving and in 20 patients the etiology was unknown. All the patients underwent vasoactive treatment (intravenous or peroral application). The treatment was most successful in patients following head injury (in 63.5% of patients the tinnitus disappeared after vasoactive treatment), but in other groups tinnitus persisted in many cases - in 80% of patients after acoustic trauma, in 78% of patients after infection, and in 75% of patients after diving. The results of audiological tests in children with tinnitus were very variable. The tinnitus patients with head injuries usually had a large hearing loss (amounting to 35 – 80 dB across the whole frequency range) and unrecordable or very small otoacoustic emissions. In other tinnitus groups, hearing loss did not exceed 20 dB in any ear and evoked otoacoustic emissions (TEOAE, DPOAE) were either equal to or only slightly smaller than those measured in age-matched controls. SOAE were present in 1/3 of ears regardless of the tinnitus group. The results of audiological tests in children with tinnitus demonstrate only a limited correlation with the origin of the tinnitus.

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P90

The meaning of specific autoimmune antibodies in occurrence and prognosis of acute inner ear disorders

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Introduction: By now the exact pathogenesis of the different forms of acute inner ear disorders like sensorineural hearing loss, sudden deafness, tinnitus and Meniere`s disease is still unknown. Disorders of inner ear blood circulation are widely discussed just like viral infections and autoimmune processes. The aim of this study is to examine specific autoimmune antibodies and their function in occurrence, therapy and prognosis of acute inner ear disorder.
Material and Methods: Prospective clinical study with n=40 patients, among 12 female and 28 male, suffering from acute inner ear disorder without any known autoimmune disease. Average age 47.6 years. Patients underwent a therapy consisting of steroids and rheologica. Hearing was examined regularly using pure-tone audiometry. Specific autoimmune antibodies were examined at the beginning and at the end of the therapy using enzyme-linked-immunosorbent assay and immuno-fluorescene-test.
Results: In 71% of all patients there was an occurrence of autoimmune antibodies, in 29% no autoimmune process could be provided. Antibodies against sarcolemma (ASA) and sinusoids were mostly detected (23%), followed by anti-nuclei-antibodies (ANA; 16%), antibodies against Microsomes, Phospholipids, Laminin (6.5% each), and anti-Endothelium and anti-Smooth muscle antibodies (3.2%). Hearing improvement after therapy was significantly higher in patients being negative in autoimmune antibodies compared to the positive ones.
Conclusion: As described before we do also agree in our study that occurrence of autoimmune antibodies should be considered as one possible mechanism in the pathogenesis of acute inner ear disease. These findings do not affect the choice of therapeutic treatment. More likely, the clinical value seems to be found especially in regard to the prognosis of disease. Studies going in depth with these findings are continued.

P91

The relationship between dental overbite and eustachian tube dysfunction

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The purpose of this study was to investigate the association between deep dental overbite and eustachian tube dysfunction (ETD). Design: Case - control study Setting: hospitalized patients in otolaryngology department at 5 Hospitals in Tehran, Iran. Patients: 132 patients between the ages of 2 and 6 years. Study Measurements: Dental overbite, overjet, and occlusal relationships were measured by one observer. ETD was defined as having ventilation tubes in place or having the recommendation for ventilation tube placement and adenoidectomy by an attending pediatric otolaryngologist. In addition, demographic information and medical and social histories were prospectively recorded. Results: In a multivariate logistic regression model, children with deep bites were 10.6 times more likely to have ETD than those without deep bites ($P < .05$). Other independent risk factors for ETD identified in this model were family history of otitis media (OM), daycare exposure, and non-breast-feeding. Conclusions: Children with deep dental overbites are at a significantly increased risk for developing ETD.

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Autoanamnesis system HearScan

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Because of the new European Union (2003/10/EY) Noise directive the methods in noise prevention at workplaces has to be changed. This will cause new actions especially in following the noise workers hearing throughout their work period. According to the directive, information of the workers hearing and risk factors to the hearing must be collected systematically and regularly while they visit work safety doctors. Furthermore they must calculate the lifetime noise dosage for every worker. For fulfilling the requirements of the new noise directive, we created the new autoanamnesis system called HearScan. By using this program, occupational healthcare physicians can swiftly check the factors that affect the workers hearing. Knowing what causes the hearing problem the doctors can direct workers more accurately in using the hearing protection devices and minimizing their noise exposure at work places.
Also an internet form was created, which makes possible for the workers to put their noise data to the HearScan database at home, before seeing the doctor. This option will save the doctor’s time during the examination of the workers significantly, because in this way the data are already in the database.

P93

Internet-based peer support system for Menière's disease

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Menière's disease (MD) is quite common. In Finland, the prevalence of MD is estimated to be 513/100 000 [1]. MD is known to affect the quality of life in a significant number of those who experience them because of the functional limitations. At worst, MD can cause depression and even isolation. Therefore, it is necessary to interfere with the course of disease.
Peer support is one of the best ways to help Menière patients to cope with MD. In the peer support is given, for example, information of the disease and ways to relieve its symptoms. Furthermore, it gives an opportunity to share experiences of the disease. With Internet-based peer support system we can offer help and information for more people. The aim of the peer support system is to modify patient's attitude towards his disease and, thus, improve the quality of life.
The peer support system consists of ten sessions. First is collected information of the patient's symptoms. Each user of the system fills in questionnaire and, based on his answers, the system searches the most similar cases and suggests them to be his live peer supports. Besides, the system uses answers in forthcoming sessions. The sessions include, among others, self conficende, positive experiences associated with MD and future health planning.
The Internet-based peer support system is created with help of Finnish Menière Society and its members. Other participants of the project are University of Tampere, Tampere University Hospital, Helsinki University Central Hospital, University Hospital of Wales and Finnish Institute of Occupational Health. We have started project by collecting data with questionnaires sent to randomly selected members. Data will be used in creation of Menière profiles that will be a basis of the system.

[1] Havia M. Menierè's Disease Prevalence and Clinical Picture [dissertation]. Helsinki, 2004.

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Hereditary factors and questionnaire-based analysis for presbyacusis

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Introduction: Aim of this study was to identify hereditary factors behind presbyacusis. For prognosis it is important to find out if there are hereditary risk factors behind the hearing loss. It is recommended to collect family history from three generations.
Methods: Self-reported questionnaires were collected from 1548 patients (mean age 75 years) who came to hearing aid fitting to Tampere University Hospital. Questionnaire included four different questions associated to hereditability. Information from patient relatives, parents, siblings and children with hearing loss (HL) at the early-onset was collected. Patients with ear surgery and also patients who did not know their family history were excluded from the study. After exclusions there were 1194 cases left for statistical analysis. Analysis was made with SPSS 14.0 for Windows.
Results: In screening HL, the hereditability of HL correlated with question on presence of blood relatives with HL and also siblings with HL. These two questions measured in somewhat different aspects of hereditability. The question concerning early onset of HL among father or mother did not correlate to hereditability. The level of HL among patients with hereditary HL did not differ from other patients but the patients with hereditary HL were on average 10 years younger than other patients. Utilization degree of hearing aids was higher in a group of patients with hereditary HL and they also found usage of current hearing aid more worthwhile than other patients.
Conclusion: It seems, that hereditary hearing loss appear earlier than other hearing losses. Patients with hereditary hearing loss use more hearing aids and feels that hearing aids really help them to survive in their everyday life. In queering hereditability, parents HL seems to be non-specific and other questions should be asked.

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